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Dated 11 November 1999

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P01/7700 0.00 - 9828217.1

Your Reference: B45168

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The Patent Office

Request for grant of a Patent

Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: VACCINE

❷ Applicant's details

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2a If you are applying as a corporate body please give:
Corporate Name: Université Libre de Bruxelles

Country *(and State of incorporation, if appropriate)*: Belgium

2b If you are applying as an individual or one of a partnership please give in full:

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Country: Belgium

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05663620002

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OR

please give details below

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Agent's address SmithKline Beecham
Corporate Intellectual Property
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Agent's ADP number 041223132061

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5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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15(4) (Divisional) ☐ 8(3) ☐ 12(6) ☐ 37(4) ☐

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Please give the date in all number format, for example, 31/05/90 for 31 May 1990

Country of Filing	Priority application number *(if known)*	Filing Date *(day, month, year)*

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- an applicant, or
- any applicant is a corporate body.

7. Are you (the applicant or applicants) the sole inventor or the joint inventors?

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Yes ☐ No ☒ ➲ ***A Statement of Inventorship on Patents form 7/77 will need to be filed (see Rule 15).***

❽ Checklist

❽ *Please supply duplicates of claim(s), abstract, description and drawings).*

8a Please fill in the number of sheets for each of the following types of document contained in this application.

Continuation sheets for this Patents Form 1/77	–
Claim(s) 3	Description 31
Abstract	Drawing(s) 27

1 Sept.

8b Which of the following documents also accompanies the application?

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Priority documents *(please state how many)*	–
Translation(s) of Priority documents *(please state how many)*	–
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Patents Form 9/77 - Preliminary Examination Report	–
Patents Form 10/77 - Request for Substantive Examination	–

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I/We request the grant of a patent on the basis of this application.

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VACCINE

FIELD OF INVENTION

This invention relates to a general method for detecting pathogenic strains of bacteria which harbour a type III secretion system, and characterising regions of the chromosome of said strain where virulence genes reside. More particularly, this invention relates to the method as applied to the pathogen *Bordetella pertussis*. Furthermore, the invention relates to newly identified polynucleotides within these regions, virulent polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly the polynucleotides and polypeptides of the present invention relate to the BopN, Orf1, Orf2, Orf3, Orf4, Orf5, Orf6, Orf7, Orf8, Orf9, Orf10, Orf11, Orf12, Orf13, Orf14, and Orf15 effector proteins of *Bordetella pertussis*.

BACKGROUND OF THE INVENTION

Type III secretion systems:

Pathogenic bacteria invade many different niches in a broad host range and cause a wide variety of syndromes. It is due to this fact that it was believed previously that each disease might be induced by a distinct molecular mechanism. However, the spectrum of such mechanisms is not as broad as first imagined; rather, bacteria exploit a number of common molecular tools to achieve a range of goals. Among these tools are type III secretion systems, which provide a means for bacteria to target virulence factors directly at host cells. These factors then tamper with host cell functions to the pathogens' benefit.

The type III export system is responsible for secretion of *Salmonella* and *Shigella* invasion and virulence factors, Enteropathogenic *Escherischia coli* (EPEC) signal transduction molecules, virulence factors in several plant pathogens (for instance *Xanthomonas campestris pv. vesicatoria* [Fenselau et al., 1992]) and Yops proteins in *Yersinia*. Yops export mechanism has been the most intensively investigated type III

secretion apparatus (see for instance: Allaoui et al., 1994; Bergman et al., 1994). In this system, more than 20 different Ysc/Lcr proteins, all encoded by the virulence plasmid pYV, are presumed to compose a secretion channel spanning the *Yersinia* cell envelope. Besides these elements involved in the secretion machinary, the pYV plasmid codes for the Yops proteins which are the secreted substrates and appear as the actual effectors of virulence.

Comparative studies of type III secretion systems originating from different species reveal that the components of the secretion machinery are strikingly conserved (Gygi *et al.*, 1995; Bogdanove *et al.*, 1996). In addition, homologs have been found in determinants which take part in flagellar assembly, indicating that this secretion pathway may be involved in surface organelle biosynthesis (Ramakrishnan *et al.*, 1991).

In contrast, however, the secreted substrates share no similarities, except in few cases. Therefore, the abandoned concept of a distinct molecular mechanism corresponding to each disease could reappear at the level of effector proteins.

Pathogenicity island

Pathogenicity islands have emerged as a novel theme in the field of bacterial virulence. Although they can comprise type III secretion systems they do not exlusively do so.

Early in the search for virulence genes, it was observed that many of these genes resided on plasmids. However, numerous virulence genes were also found on the chromosome. Surprisingly, similarly as when they are found on plasmids, the chromosomal virulence genes often clustered in functionally related groups also. Such groups of virulence genes gave rise to the concept of pathogenicity islands (Pais) which can be defined as compact, distinct genetic units carrying virulence genes. These units,

often flanked by direct repeats, occupy large chromosomal regions (often > 30 kb) and are present in pathogenic strains, whilst being absent or sporadically distributed in less-pathogenic (or non-pathogenic) strains of a bacterial species. These DNA segments are frequently associated with tRNA genes and/or insertion sequence (IS) elements at their boundaries. In addition, their G+C content often differs from that of host bacterial DNA, suggesting a foreign origin.

Pathogenicity islands have been discovered in an increasing number of bacterial pathogens, including different categories of *E. coli, Salmonella typhimurium, Yersinia* spp, *Helicobacter pylori*, *Vibrio cholera* etc.

The first intensively studied pathogenicity islands were Pai I and Pai II, which encode the haemolysin determinants of uropathogenic *E. coli*. These two Pais, are flanked by direct repeats and can be deleted from the chromosome at frequencies of 10^{-4}, resulting in non-virulent mutant strains. Another pathogenicity island of 35 kb has recently been identified on the chromosome of enteropathogenic *E. coli* (EPEC) and was found to encode all known determinants involved in the so-called "attaching and effacing" (AE) lesion formation. This region was therefore referred to as "locus of enterocyte effacing" (LEE). Despite the fact that uropathogenic and enteropathogenic *E. coli* cause completely different infectious diseases, Pai I of the uropathogenic strains and the LEE locus of EPEC are inserted at exactly the same positions into the *E. coli* chromosome.

While some authors support a definition of pathogenicity islands which necessarily includes its chromosomal location, others have extended the concept to blocks of virulence genes, regardless of their location in chromosomes, plasmids or phages. The fact that, on one hand, phages and plasmids can easily insert into and excise from the chromosome and, on the other, that cryptic origins of plasmid replication, or phage related sequences were detected in Pais, prompted us to adopt the latter and less restrictive definition.

The pathogenicity islands (PAIs) which code for a type III secretion system encompass genes that divide into two classes, I and II. Class I encompasses the genes coding for the secretion machinery components and their regulators of expression, class II encompasses the genes encoding secreted effector proteins. Both *Yersinia lcrD* and *yscU* belong to class I. The precise functions of class I determinants is not well understood. Although it is sometimes not straightforward to make a clear distinction between class I and class II components, genes of class I can be identified as being present in many different species, and a comparison of their respective gene sequences indicate that equivalent genes share a significant (*yscI*, *yscO*) or even high level (*lcrD*, *yscU*, y*scN*) of sequence similarity (Hueck, 1998).

The second class of genes (class II) codes for proteins which constitute the substrate secreted by the translocon. These proteins appear as the actual effectors of virulence and are referred to as target proteins, virulence effector proteins or, simply, effectors. In contrast to the situation prevailing in class I gene products, the effectors share no, or very weak, similarities between species. Effector proteins are those which present the best biological, vaccine and diagnostic potentialities.

The inventors have realised that the clustering of class I and class II genes inside a single pathogenicity island, offers the opportunity of conveniently finding and characterising unknown class II genes by targeting class I genes which can be identified using a known sequence of one of their numerous orthologues.

Bordetella pertussis

Whooping cough is a disease caused by infection by *Bordetella pertussis*, and is a serious and debilitating human disease particularly in young children. Although whole cell and acellular vaccines are available that are effective against the disease, there

remains a need for the identification of further highly purified pertussis proteins that could be used in a more efficacious pertussis vaccine.

Although many pertussis virulence associated factors are known such as pertussis toxin, filamentous haemagglutinin, pertactin, which have been included in various acellular vaccines, there is no convenient genetic method for identifying further virulence factors using the pertussis genome (short of laboriously sequencing the whole genome). Although class I type III secretion system virulence genes have recently been shown to exist in *B. bronchiseptica* and *B. pertussis* (Yuk *et al.*, 1998), there has been no complete analysis of a pathogenicity island in *Bordetella*, and the identity and characterisation of effector genes within such a pathogenicity island has been unknown up until the present invention.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to a method for the identification of new virulence genes in bacterial strains containing a type III secretion system. In particular, the invention allows the identification of the effector virulence genes associated within a pathogenicity island containing the genes for the type III secretion system. Another aspect of the invention a method for the identification of pathogenic bacterial strains containing a type III secretion system. Another aspect of the invention relates to *Bordetella pertussis* BopN, Orf1, Orf2, Orf3, Orf4, Orf5, Orf6, Orf7, Orf8, Orf9, Orf10, Orf11, Orf12, Orf13, Orf14, Orf15 effector proteins, and the respective polynucleotide sequences encoding them.

Although the general concepts of type III secretion systems and pathogenicity islands have been reported, the problem of how simply and reliably to identify whether any given organism has such cell machinery has not been accomplished until now. Such a method is extremely useful to establish whether a given strain has a type III secretion

system within a pathogenicity island, to characterise unknown virulence genes within the pathogenicity island, and to use in quick diagnostic methods for determining whether a cultured bacterial strain containing a type III secretion system is pathogenic.

In the present invention, a novel, general method is described to achieve the above aims. More specifically, the invention utilises a method that employs ideally-suited primers designed specifically from the sequence of the virulent *Yersinia enterocolitica lcrD* gene as a target sequence. The presence of a type III secretion system within a pathogenicity island in *Bordetella pertussis* was discovered, and every gene within the pathogenicity island was characterised.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Nucleotide and deduced amino acid sequences of the cloned 152 bp amplicon. The primers involved in the original amplification, the subsequent nested PCR, and the gene library screening are all derived from this sequence, and listed specifically in Table 1.

Fig. 2. PileUp figure from the deduced amino acid sequences homologous to *Yersinia* LcrD. Abbreviations: BbuFlhA = *Borrelia burgdorferi* FlhA; TpaFlhA = *Treponema pallidum* FlhA; BsuFlhA = *Bacillus subtilis* FlhA; CjeFlbA = *Campylobacter jejuni* FlbA; HpyFlhA = *Helicobacter pylori* FlhA; EcoFlhA - *Escherichia coli* FlhA; StyFlhA = *Salmonella typhimurium* FlhA; YenFlhA = *Yersinia enterocolitica* FlhA; PmiFlhA = *Proteus mirabilis* FlhA; CcrFlbF = *Caulobacter crescentus* FlbF; EcoFhiA = *Escherichia coli* FhiA; EamHrpI = *Erwinia amylovora* HrpI; PsyHrpI = *Pseudomonas syringae* HrpI; ECEPSepA = Enteropathogenic *Escherichia coli* SepA; StySsaV = *Salmonella typhimurium* SsaV; RsoHrpO = *Ralstonia solanacearum* HrpO; XcaHrpC2 = *Xanthomonas campestris* HrpC2; SflMxiA = *Shigella Flexneri* MxiA; StyInvA = *Salmonella typhimurium* InvA; PaePcrD = *Pseudomonas aeruginosa* PcrD; YenLcrD =

Yersinia enterocolitica LcrD; BpeBcrD = *Bordetella pertussis* BcrD; CpsTtsB = *Chlamydia psittaci* TtsB.

Fig. 3. Organization of the *Bordetella pertussis* pathogenicity island (Pai). Four house keeping genes (hatched boxes) and the transposase gene of IS*481* (black box) are surrounding the Pai. The Pai consists of genes coding for determinants involved in the secretory apparatus and its regulation (class I genes, in grey boxes) as well as ORFs which putitively code for effector proteins (class II genes, in white boxes). Letters indicate the respective class I *bsc* genes whereas numbers correspond to the class II ORFs listed in Table 3.

Fig. 4. PileUp figure from the deduced amino acid sequences homologous to *Yersinia* YscU. Abbreviations: BbuFlhB = *Borrelia burgdorferi* FlhB; TpaFlhB = *Treponema pallidum* FlhB; EcoFlhB - *Escherichia coli* FlhB; StyFlhB = *Salmonella typhimurium* FlhB; PmiFlhBpart = partial *Proteus mirabilis* FlhB; YenFlhB = *Yersinia enterocolitica* FlhB; BsuFlhB = *Bacillus subtilis* FlhB; HpyFlhB = *Helicobacter pylori* FlhB; AtuFlhB = *Agrobacterium tumefaciens* FlhB; CcrPodW = *Caulobacter crescentus* PodW; SflSpa40 = *Shigella flexneri* Spa40; StySpaS = *Salmonella typhimurium* SpaS; EcoEscU = *Escherichia coli* EscU; StySsaU = *Salmonella typhimurium* SsaU; BpeBscU = *Bordetella pertussis* BscU; YenYscU = *Yersinia enterocolitica* YscU; RsoHrpN = *Ralstonia solanacearum* HrpN; XcaOrf0part = partial *Xanthomonas campestris* Orf0; EamHrcU = *Erwinia amylovora* HrcU; EheHrcUpart = partial *Erwinia herbicola* HrcU; PsyHrpY = *Pseudomonas syringae* HrpY; CpsOrf1 = *Chlamydia psittaci* Orf1.

Fig. 5. The DNA sequence of the Bordatella pertussis genome comprising the type III secretion system pathogenicity island. Reference should be made to tables 2, 3, and 4 and Fig. 3 for information regarding open reading frames.

DESCRIPTION OF THE INVENTION

Type III secretion systems identified to date are encoded by either chromosal or plasmidic pathogenicity island genes. However, no where in the prior art was it realised that the conservation of genes encoding class I components of type III secretion systems and the clustering of these genes with effector protein coding sequences offered the opportunity for detecting unidentified target proteins involved in host colonisation. Such proteins would be potentially valuable in both vaccinal and diagnostic fields.

Although the known sequence of a gene encoding any conserved (class I) type III secretion machinery protein can be used in performing this invention, the *lcrD* gene is preferred. The chosen gene will act as a target for detecting unidentified pathogenicity islands in related bacterial species. The *lcrD* gene from *Yersinia* is preferred as it codes for the archetype of the recently identified LcrD/FlbF family of proteins. Members of this family are involved in host cell invasion, virulence in several phytopathogenic bacteria, or in flagellar assembly. *lcrD* is preferred because the LcrD protein, and consequently the gene encoding it, is one of the most conserved determinants of the secretion machinary. Additionally, multiple amino acid comparisons have shown that the classification of the LcrD family members can be split into two main subfamilies, which, interestingly, can be correlated with the functions assigned to these proteins of each subfamily. One subfamily encompasses all the motility-involved proteins, while the other encompasses all the virulence-related determinants. This observation is illustrated in Fig. 2 (and mentioned in Gyri *et al.* (1995) & Bogdanove *et al.* (1996)). Thus, if an unknown *lcrD* homologous gene is identified, it may, after being routinely sequenced, be classified as a virulence or a flagellar gene. Once the pathogenicity island is identified, this simple test would therefore define whether the search for other virulence genes on the pathogenicity islands should be initiated.

The preferred method for identifying unknown pathogenicity islands comprising a type III secretion system is by:

i) identifying two highly conserved regions of the target protein sequence (preferably of LcrD). Preferably, both regions should contain conserved amino acids which are encoded by the fewest number of codon possibilities e.g. Methionine (ATG being the only possibility) or Tryptophan (TGG being the only possibility). This minimises the number of permutations in both degenerate primer sets that are designed in the next stage of the process, thus ensuring a greater probability that each primer set will specifically anneal to the unknown *lcrD*-equivalent gene (thereby minimising background non-specific interactions). Most preferably, regions should also be chosen that are clearly distinguishable from the paralogue *flhA* flagellar genes, present in all flagellated bacterial strains.

ii) designing a degenerate set of primers for both of the chosen regions such that a) the primers are at least 15 bases long, preferably 20-30 bases long, and still more preferably 21-23 bases long, b) they are degenerate at bases that can be more than one type of nucleotide whilst still encoding the same amino acid (due to the degeneracy of codon usage for amino acids), but no more degenerate than is required to cover all permutations for the amino acid region selected, and c) the primer set that encodes the more N-terminal region of the chosen protein should correspond to the coding strand of its corresponding double-stranded DNA sequence, and the set that encodes the more C-terminal region should correspond to the complementary strand of the corresponding double-stranded DNA sequence.

iii) synthesising the degenerate primer sets of step ii) using conventional DNA synthesis methods well known in the art.

iv) purifying the primer sets of step iii)

v) adding both the primer sets and a sample containing nucleic acid from a bacterial strain (preferably a cell sample of the bacterial species itself) together in appropriate quantities and in an appropriate buffer in order to perform a polymerase chain reaction (PCR)

vi) performing a PCR reaction in order to amplify the region of the gene between the two primers (conditions for performing the PCR reaction can be optimised using techniques well known in the art)

vii) observing the reaction products on a gel (preferably an agarose gel) for an amplified product of the size expected; if no such product is present, the bacterial strain is unlikely to use a type III secretion system; if such a product is present, the bacterial strain is likely to have a type III secretion system, and is likely to be pathogenic.

The preferred method for confirming that the amplified product actually corresponds to a virulence gene is by carrying out steps i)-vii) above (where the target protein is LcrD) and then:

viii) optionally separating the product of correct size from any background products of incorrect size by removing the correct band from the gel, purifying the product by conventional means, and amplifying the product once more with the two degenerate primer sets in another PCR reaction (under preferably more stringent PCR conditions) [this step is required should the product of step vii) not be pure enough for direct cloning].

ix) inserting the DNA fragment by conventional means into a vector which is capable of being sequenced, and sequencing the fragment.

x) comparing the deduced amino acid sequence of ix) with that of known members of the LcrD/FlbF family of proteins to associate the amplified product as being part of either a virulence or a flagellar gene.

And optionally:

xi) using the internal sequence of the fragment to design primers that are the exact sequence of, and specific to, the unknown *lcrD*-equivalent gene.

xii) using the primers of xi) firstly to screen a genomic library of the organism for positive clones.

xiii) isolating the clones of xii), and sequence one or more of said clones.

xiv) scanning the sequence of one clone (and overlapping sequences of other clones) to search for an open reading frame which is approximately the same size as *lcrD* (approximately 2100bp), and encodes a protein homologous to LcrD

xv) ascertaining whether the LcrD-equivalent protein is more homologous with the *flbF* (flagellar protein secretion) gene family or the *lcrD* (type III secretion system pathogenicity island) gene family.

The preferred method for characterising the whole pathogenicity island and defining unidentified virulence effector genes is by carrying out steps i)-xv) above (where the target protein is LcrD) and then:

xvi) if the sequence is more homologous with the *lcrD* gene family, designing primers at either extreme of the gene sequence already ascertained, and scanning and sequencing the genomic library (using a standard chromosome walking strategy – where the insert boundaries of an original clone serves as a probe for screeing and cloning adjacent regions) to sequence eventually the whole of the pathogenicity island (both boundaries of which will be defined by the presence of either direct or inverted repeats, or insertion sequences, or the presence of house-keeping genes)

xvii)defining unidentified virulence effector genes within the sequenced pathogenicity island

xviii)cloning, expressing and characterising the virulence genes of xvii) which encode virulence effector proteins of the organism

Definitions

"Pertussis pathogenicity proteins" refers generally to polypeptides having the amino acid sequence encoded by the genes defined in tables 2 and 3, or an allelic variant thereof. These proteins are: BcrD, BcrH, BscC, BscD, BscE, BscF, BscI, BscJ, BscK, BscL, BscN, BscO, BscP, BscQ, BscR, BscS, BscT, BscU, BscV, BrpL, BopN, Orf1, Orf2, Orf3, Orf4, Orf5, Orf6, Orf7, Orf8, Orf9, Orf10, Orf11, Orf12, Orf13, Orf14, Orf15.

"Pertussis pathogenicity genes" refers to polynucleotides having the nucleotide sequence defined in tables 2 and 3, or allelic variants thereof and/or their complements.

These genes are: *bcrD, bcrH, bscC, bscD, bscE, bscF, bscI, bscJ, bscK, bscL, bscN, bscO, bscP, bscQ, bscR, bscS, bscT, bscU, bscV, brpL, bopN, orf1, orf2, orf3, orf4, orf5, orf6, orf7, orf8, orf9, orf10, orf11, orf12, orf13, orf14, orf15.*

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslational natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND

MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference

polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions (preferably conservative), additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis. Variants should retain one or more of the biological activities of the reference polypeptide. For instance, they should have similar antigenic or immunogenic activities as the reference polypeptide. Antigenicity can be tested using standard immunoblot experiments; preferably using polyclonal sera against the reference polypeptide. The immunogenicity can best be tested by measuring antibody responses (using polyclonal sera generated against the variant polypeptide) against purified reference polypeptide in a standard ELISA test. Preferably, a variant would retain all of the above biological activities.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heijne, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New

York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., *et al.*, *Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al.*, *J Molec Biol* (1990) 215:403). Most preferably, the program used to determine identity levels was the GAP program, as was used in the Examples below.

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include on average up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the invention

In one aspect, the present invention relates to Bordetella pathogenicity proteins (or polypeptides). The Bordetella pathogenicity polypeptides include the polypeptides encoded by the genes defined in tables 2 and 3; as well as polypeptides comprising the amino acid sequence encoded by the genes defined in tables 2 and 3; and polypeptides comprising the amino acid sequence which have at least 75% identity to that encoded by the genes defined in tables 2 and 3 over their entire length, and preferably at least 80% identity, and more preferably at least 90% identity. Those with 95-99% identity are highly preferred.

The Bordetella pathogenicity polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It may be advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the Bordetella pathogenicity polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that is the same as part, but not all, of the amino acid sequence of the aforementioned Bordetella pathogenicity polypeptides. As with Bordetella pathogenicity polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of Bordetella pathogenicity polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of Bordetella pathogenicity polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus and/or transmembrane region or deletion of two continuous

series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate Bordetella pathogenicity protein activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity (for instance antigenic or immunogenic) of the Bordetella pathogenicity protein, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination. Most preferred variants are naturally occurring allelic variants of Bordetella pathogenicity polypeptide present in strains of *Bordetella pertussis*.

The Bordetella pathogenicity polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the invention

Another aspect of the invention relates to Bordetella pathogenicity polynucleotides. Bordetella pathogenicity polynucleotides include isolated polynucleotides which encode the Bordetella pathogenicity polypeptides and fragments respectively, and polynucleotides closely related thereto. More specifically, Bordetella pathogenicity polynucleotides of the invention include a polynucleotide comprising the nucleotide sequence of genes defined in table 2 or 3, encoding a Bordetella pathogenicity polypeptide. Bordetella pathogenicity polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 75% identity over its entire length to a nucleotide sequence encoding the Bordetella pathogenicity polypeptide encoded by the genes defined in tables 2 and 3, and a polynucleotide comprising a nucleotide sequence that is at least 75% identical to that of the genes defined in tables 2 and 3. In this regard, polynucleotides at least 80% identical are particularly preferred, and those with at least 90% are especially preferred. Furthermore, those with at least 95% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under Bordetella pathogenicity polynucleotides is a nucleotide sequence which has sufficient identity to a nucleotide sequence of a gene defined in tables 2 and 3 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such Bordetella pathogenicity polynucleotides.

The nucleotide sequence encoding Bordetella pathogenicity polypeptide encoded by the genes defined in tables 2 and 3 may be identical to the polypeptide encoding sequence contained in the genes defined in tables 2 or 3, or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide encoded by the genes defined in tables 2 and 3 respectively.

When the polynucleotides of the invention are used for the recombinant production of Bordetella pathogenicity polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding

sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro-protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al.*, *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag, or is glutathione-s-transferase. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding Bordetella pathogenicity protein variants comprising the amino acid sequence of the Bordetella pathogenicity polypeptide encoded by the genes defined by tables 2 and 3 respectively in which several, 10-25, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Most preferred variant polynucleotides are those naturally occurring *Bordetella pertussis* sequences that encode allelic variants of the Bordetella pathogenicity proteins in *B. pertussis*.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence of any gene defined in tables 2 and 3 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding Bordetella pathogenicity polypeptides respectively and to

isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than *Bordetella pertussis*) that have a high sequence similarity to the Bordetella pathogenicity genes. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides. In one embodiment, to obtain a polynucleotide encoding Bordetella pathogenicity polypeptide, including homologs and orthologs from species other than *Bordetella pertussis*, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having a nucleotide sequence contained in one of the gene sequences defined by tables 2 and 3, or a fragment thereof; and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Thus in another aspect, Bordetella pathogenicity polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having a nucleotide sequence contained in one of the genes defined by table 2 and 3, or a fragment thereof. Also included with Bordetella pathogenicity polypeptides are polypeptides comprising amino acid sequences encoded by nucleotide sequences obtained by the above hybridization conditions. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Diagnostic Assays

This invention also relates to the use of Bordetella pathogenicity polypeptides, or Bordetella pathogenicity polynucleotides, for use as diagnostic reagents. Detection of Bordetella pathogenicity polypeptides will provide a diagnostic tool that can add to or define a diagnosis of *B. pertussis* disease, among others.

Materials for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly *B. pertussis* disease, which comprises:
(a) a Bordetella pathogenicity polynucleotide, preferably the nucleotide sequence of one of the gene sequences defined by tables 2 and 3, or a fragment thereof ;
(b) a nucleotide sequence complementary to that of (a);
(c) a Bordetella pathogenicity polypeptide, preferably the polypeptide encoded by one of the gene sequences defined in tables 2 and 3, or a fragment thereof;
(d) an antibody to a Bordetella pathogenicity polypeptide, preferably to the polypeptide encoded by one of the gene sequences defined in tables 2 and 3; or
(e) a phage displaying an antibody to a Bordetella pathogenicity polypeptide, preferably to the polypeptide encoded by one of the gene sequences defined in tables 2 and 3.

It will be appreciated that in any such kit, (a), (b), (c), (d) or (e) may comprise a substantial component.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with Bordetella pathogenicity polypeptide or epitope-bearing fragments, analogs, outer-membrane vesicles or cells (attenuated or otherwise), adequate to produce antibody and/or T cell immune response to protect said animal from *B. pertussis* disease, among others. In

particular the invention relates to the use of Bordetella pathogenicity polypeptides encoded by the genes defined in table 3 – the effector proteins. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering Bordetella pathogenicity polypeptide via a vector directing expression of Bordetella pathogenicity polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

A further aspect of the invention relates to an immunological composition or vaccine formulation which, when introduced into a mammalian host, induces an immunological response in that mammal to a Bordetella pathogenicity polypeptide (particularly one encoded by a gene defined in table 3) wherein the composition comprises a Bordetella pathogenicity gene, or Bordetella pathogenicity polypeptide or epitope-bearing fragments, analogs, outer-membrane vesicles or cells (attenuated or otherwise). The vaccine formulation may further comprise a suitable carrier. The Bordetella pathogenicity polypeptide vaccine composition is preferably administered orally or parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Yet another aspect relates to an immunological/vaccine formulation which comprises the polynucleotide of the invention. Such techniques are known in the art, see for example Wolff *et al.*, *Science*, (1990) *247*: 1465-8.

EXAMPLES

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Example 1: A type III secretion system is present in a pathogenicity island in *Bordetella pertussis*.

The presence of a *lcrD* homologous gene in the *Bordetella pertussis* genome was investigated by polymerase chain reaction (PCR). The primers used (oligos 95080 and 95081 shown in Table 1) were degenerate oligonucleotides corresponding to highly conserved regions of the amino acids sequences of the LcrD/FlbF family of proteins. These primers were also designed to favour the amplification of virulence genes instead of their paralogue *flhA* or *flbF* flagellar genes, present in flagellated bacterial strains. The presence of the 3' triplet CAT in oligonucleotide 95081 is a determinant – indeed when multiple sequence analysis is done using known homologous sequences (database searching was done with either the FASTA and TFASTA programs of the GCG9 package, or with BLASTN, BLASTP and BLASTX programs, and alignments were carried out with the PILEUP program from the GCG9 package) it could be seen that the CAT triplet codes for a methionine which is exclusively present in virulence sequences while absent in the flagellar ones.

When analysed on agarose gel, the PCR product appeared as a heterogeneous mix of fragments, one of which was presenting the expected size (around 150 bp). A second round of amplification using the approximately 150 bp DNA as template yielded a single amplicon which was cloned in pCRII (obtained from Invitrogen) for further characterisation. It appeared as a 152 bp fragment whose nucleotide sequence (Fig. 1),

although similar to all *lcrD/flbF* homologous genes, shares a higher level of identity with the virulence (*lcrD*-like) genes.

Table 1.

oligonucleotides	sequence[1]	features	*lcrD* corresponding codons[2]
95080	GSH ATG CCW GGH AAR CAR ATG	direct, degenerate	150 to 156
95081	GC RTC DCC YTT DAC RAA YTT CAT	complement, degenerate	193 to 200
95363	CC ATC GAC GCG GAC TTG CGC G	direct, non-degenerate	157 to 164
95364	CGC GCC GTC CAT GGC GCC ATA	complement, non-degenerate	186 to 192
96110	C CGA CGC CGA CGC CGT ACG GTC	direct, non-degenerate	172 to 179

[1] The letter code for nucleotide ambiguity proposed by IUB (Nomenclature Committee, 1985, Eur. J. Biochem., **150**: 1-5) was used.

[2] The DNA sequence of the *lcrD* gene from *Yersinia enterocolitica* used for this work was published by Plano *et al.* (1991).

To ensure that the cloned fragment was actually a *B. pertussis* sequence PCR was performed under stringent conditions with serial 10-fold dilutions of DNA from *B. pertussis*. The optimisation of stringent PCR conditions require a perfect match between template and primers. It was likely, however, that due to the degeneration of the original primers, the 152 bp sequence initially obtained had, at its boundaries, a few base pair differences with the actual *B. pertussis lcrD*-like (hereafter called *bcrD*) sequence. A nested PCR approach using internal primers (oligos 95363 and 95364 Table 1) was therefore preferred, as primers known to be the correct *B. pertussis* sequence are used. A dose-response-relationship was observed between the 10-fold dilutions of *B. pertussis* template DNA and the product of the nested PCR, suggesting that the 152 bp amplicon actually originates from the *Bordetella* genome.

Comparison of the 152 bp sequence with *lcrD/flbF* genes allowed us to define a specific DNA stretch (oligo 96110 in Table 1) which was used as a probe for screening a genomic library of *B. pertussis* constructed in the plasmid vector pBR327 (Delisse-Gathoye *et al*, 1990, *Infect-Immun.* **58**: 2895-905). Several positive clones were isolated and restriction analysis of their resident plasmids showed that they harboured overlapping inserts. The entire nucleotide sequence of one insert was determined, revealing a large open reading frame (ORF). This 2100 bp ORF encoded a 75 kDa polypeptide which is 59 % and 47 % identical to the yersinial proteins LcrD and FlhA respectively. Multiple amino acids comparisons of all known members of the LcrD/FlbF family of proteins, including the *B. pertussis* BcrD deduced amino acid sequence, showed that this sequence clearly ranked within the virulence associated determinants (Fig. 2). These data strongly suggest that *B. pertussis* possesses a type III export system, involved in the secretion of virulence effectors.

The *B. pertussis lcrD*-like nucleotide sequence (*bcrD*) has been submitted to EMBL and assigned the accession number Y13383.

This general technique has been useful for determining the presence/absence of a type III secretion system in other bacterial strains. The human pathogens *Borrelia burgdorferi* and *Helicobacter pylori* were intensively screened for such a system using this technique. No evidence for a type III secretion system could be found. The subsequent publication of the genome sequences of these microorganisms has confirmed the absence of similar systems in these species. In contrast, the method allowed the amplification of a DNA fragment from the phytopathogen *Pseudomonas corrugata*, which clearly ranks among the virulence sequences. This technique could be applied to any Gram negative pathogen of medical or agronomic importance such as *Neisseria* spp, *Moraxella catharalis*, *Vibrio cholerae*, any Enterobacteriaceae, *Pseudomonas* spp, *Haemophilus influenzae*, *Brucella* spp, *Francisella tularensis*, *Pasteurella* spp, *Legionella pneumophila*. Even in strains that have been fully sequenced, this technique

can be used as a simple method for checking alternate types or strains of the same species. For instance, some types of pathogenic *Escherichia coli* harbour a type III secretion system whereas others do not.

Example 2: Analysis of the *B. pertussis bcrD* flanking sequences to characterise the pathogenicity island and virulence-related proteins encoded therein

The tendency for systematic clustering of type III encoding genes inside pathogenicity islands prompted the analysis of *B. pertussis bcrD* flanking sequences. The whole region containing the pathogenicity island was sequenced by chromosome walking taking care to pay attention to the fact that each Pathogenicity island region must be represented in at least two independent clones, to avoid possible artefacts due to chimeric DNA inserts. This revealed clustered ORFs that could be classed in 3 categories: class I type ORFs (table 2); class II type ORFs (table 3) – the effector proteins which have the best vaccinal and diagnostic properties; & insertion sequences, and ORFs homologous to house keeping genes of other species (table 4). Although there is no general rule for defining the boundaries of a Pathogenicity island, they can be demarcated with a direct or inverse repeat at one or other boundary, however the absolute demarcation of the boundaries can only really be done by the detection of house keeping genes at the extremes of the sequence. In the present case, an insertion sequence (IS in Fig. 3) was present at the 5' end of the island (separating the virulence ORFs from the house keeping genes), but absent at the 3' end. In addition, the presence of house keeping genes (*greA* and ICFG-like) surrounding a locus which, according to sequence data, encompasses numerous virulence sequences is a good indication of the boundaries of the island. The complete gene organisation of the pathogenicity island is schematically represented in Figure 3. The precise definition of the PAI boundaries requires further experimental data, such as the characterisation of the corresponding chromosomal region of a *Bordetella* strain which is devoid of a type III secretion system.

Table 2

names	Coding sequence from/to (with reference to Fig. 5)	Coding DNA strand	Homologous genes (from *Yersinia*, unless otherwise specified)
Class I genes, i.e. genes coding for determinants involved in the secretory apparatus and their regulation			
bcrD	8656/10755	complement	*lcrD*
bcrH	14097/14582	direct	*lcrH* (= *sycD*)
bscC	26955/28757	direct	*yscC*
bscD	7379/8659	complement	*yscD*
bscE	7039/7338	complement	none
bscF	6783/7049	complement	*yscF*
bscI	17892/18218	direct	*yscI*
bscJ	18215/19039	direct	*yscJ*
bscK	19032/19694	direct	none
bscL	19664/20302	direct	*yscL*
bscN	20307/21641	direct	*yscN*
bscO	21641/22150	direct	*yscO*
bscP	22147/22695	direct	none
bscQ	22692/23771	direct	*yscQ*
bscR	23768/24439	direct	*yscR*
bscS	24445/24711	direct	*yscS*
bscT	24723/25523	direct	*yscT*
bscU	25520/26569	direct	*yscU*
bscV	26566/26964	direct	none
brpL	28778/29380	complement	*hrpL* (*Pseudomonas syringae*)

Table 3

Names	Coding sequence from/to (with reference to Fig. 5)	Coding DNA strand	Homologous genes (from *Yersinia*, unless otherwise specified)
Class II ORFs which putatively code for effector proteins			
bopN	11906/13003	complement	*YopN* (= *lcrE*)
orf1	6160/6747	direct	none
orf2	10752/11120	complement	none
orf3	11117/11527	complement	none
orf4	11532/11909	complement	none
orf5	13002/13784	direct	none
orf6	13806/14081	direct	none
orf7	14630/15571	direct	none
orf8	15601/16803	direct	none
orf9	16827/17288	direct	*bcrH*
orf10	17293/17814	direct	*pcr4* (*Pseudomonas aeruginosa*)
orf11	29412/29591	complement	none
orf12	29555/30529	complement	none
orf13	30631/31776	direct	none
orf14	31773/33005	complement	none
orf15	32370/33014	direct	none

Table 4

No name specified	Coding sequence from/to (with reference to Fig. 5)	Coding DNA strand	Homolgous sequences
Insertion Sequences and house keeping genes			
	711/2024	direct	uracil permease genes of numerous bacteria
	2055/3590	complement	chemoreceptor genes of numerous bacteria
	4220/4696	direct	*greA* (*Escherichia coli*)
	4998/5948	complement	transposase genes of numerous bacteria
	33002/34852	complement	ICFG gene (*Synechocystis* sp)

Next to the *bcrD* gene, there is an open reading frame (ORF) whose deduced amino acid sequence shares significant similarities with the YscU protein of *Yersinia* spp (39% identity and 51% similarity) and other known YscU homologs (Fig. 4). YscU, like LcrD, is a component of the *Yersinia* type III secretion machinery involved in the virulence mechanisms of the bacteria. *B. pertussis* therefore possesses a classical type III secretion system which is most probably involved in pathogenicity. This latter point can be investigated through phentoypic analyses of mutants (see below).

The total length of the Pai is approximately 30 to 40 kb. The DNA sequence of the whole region is presented in Figure 5, and is referred to in tables 2, 3, and 4.

No homologies could be found between the *B. pertussis* Class II Pai DNA sequences and the sequences reported in the GenEMBL databases (except for those stated in table 3). The expressed products of these unknown genes within the Pai responsible for virulence, will be useful in the development of a vaccine formulation against pathogenic *Bordetella pertussis*.

To address the precise function of the Pai, a *bcrD* mutant was engineered by allelic exchange. In the resulting mutant, the *bcrD* gene was disrupted by an *aphA*-3 cassette conferring kanamycin resistance. This cassette was inserted in such a way that translation was not interrupted, avoiding any polar effect on expression of putative downstream cistrons. A mutant has been isolated and its associated phenotype is being currently analysed.

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Yuk, M. H., Harvill, E. T., and Miller, J. F. (1998) The BvgAS virulence control system regulates type III secretion in *Bordetella bronchiseptica*. *Mol. Microbiol.* **28**:945-959.

We claim:

1. A method of identifying virulence genes from a pathogenicity island containing a type III secretion system from pathogenic strains of bacteria, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers of *lcrD*-like genes present in said pathogenic strain of bacteria;

sequencing the *lcrD*-like gene;

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes; and

if a virulence-associated member, sequencing the entire pathogenicity island, and identifying genes within this sequence.

2. A method of determining whether a particular bacterial strain harbours a type III secretion system involved in pathogenicity, comprising:

designing degenerate PCR primers complementary to well-conserved regions specific to the LcrD polypeptide of *Yersinia*;

amplifying the polynucleotide containing the DNA sequence between (and including the DNA sequence of) the primers to determine the presence of any *lcrD*-like genes in said bacterial strain;

if amplified successfully, sequencing the *lcrD*-like gene; and

determining whether the DNA sequence is more homologous: to the virulence-associated family of *lcrD*-like genes, or to the flagellar-associated family of *lcrD*-like genes.

3. An isolated polynucleotide comprising a nucleotide sequence encoding the BopN polypeptide of *Bordetella pertussis*.

4. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp1 polypeptide of *Bordetella pertussis*.

5. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp2 polypeptide of *Bordetella pertussis*.

6. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp3 polypeptide of *Bordetella pertussis*.

7. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp4 polypeptide of *Bordetella pertussis*.

8. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp5 polypeptide of *Bordetella pertussis*.

9. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp6 polypeptide of *Bordetella pertussis*.

10. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp7 polypeptide of *Bordetella pertussis*.

11. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp8 polypeptide of *Bordetella pertussis*.

12. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp9 polypeptide of *Bordetella pertussis*.

13. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp10 polypeptide of *Bordetella pertussis*.

14. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp11 polypeptide of *Bordetella pertussis*.

15. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp12 polypeptide of *Bordetella pertussis*.

16. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp13 polypeptide of *Bordetella pertussis*.

17. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp14 polypeptide of *Bordetella pertussis*.

18. An isolated polynucleotide comprising a nucleotide sequence encoding the Omp15 polypeptide of *Bordetella pertussis*.

19. An isolated polypeptide encoded by the polynucleotide of claims 3-18.

20. A vaccine comprising the polypeptide of claim 19.

21. A kit for diagnosing infection with *B. pertussis* bacteria in a human comprising a polynucleotide of claims 3-18 or a polypeptide of claim 19.

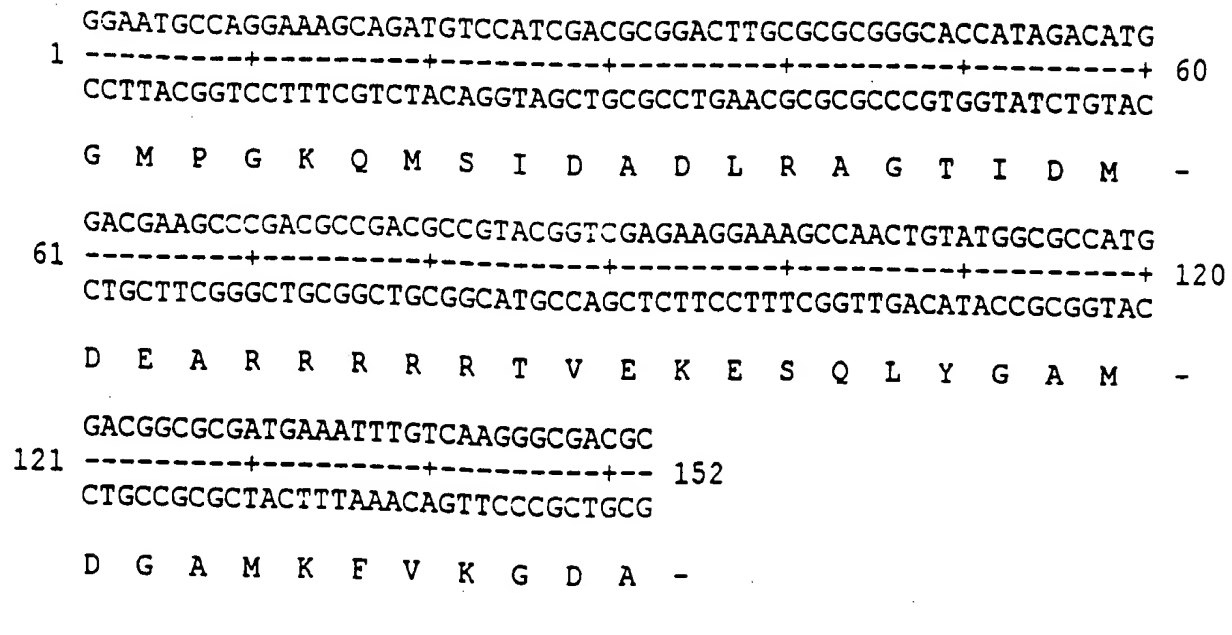
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Fig. 2A

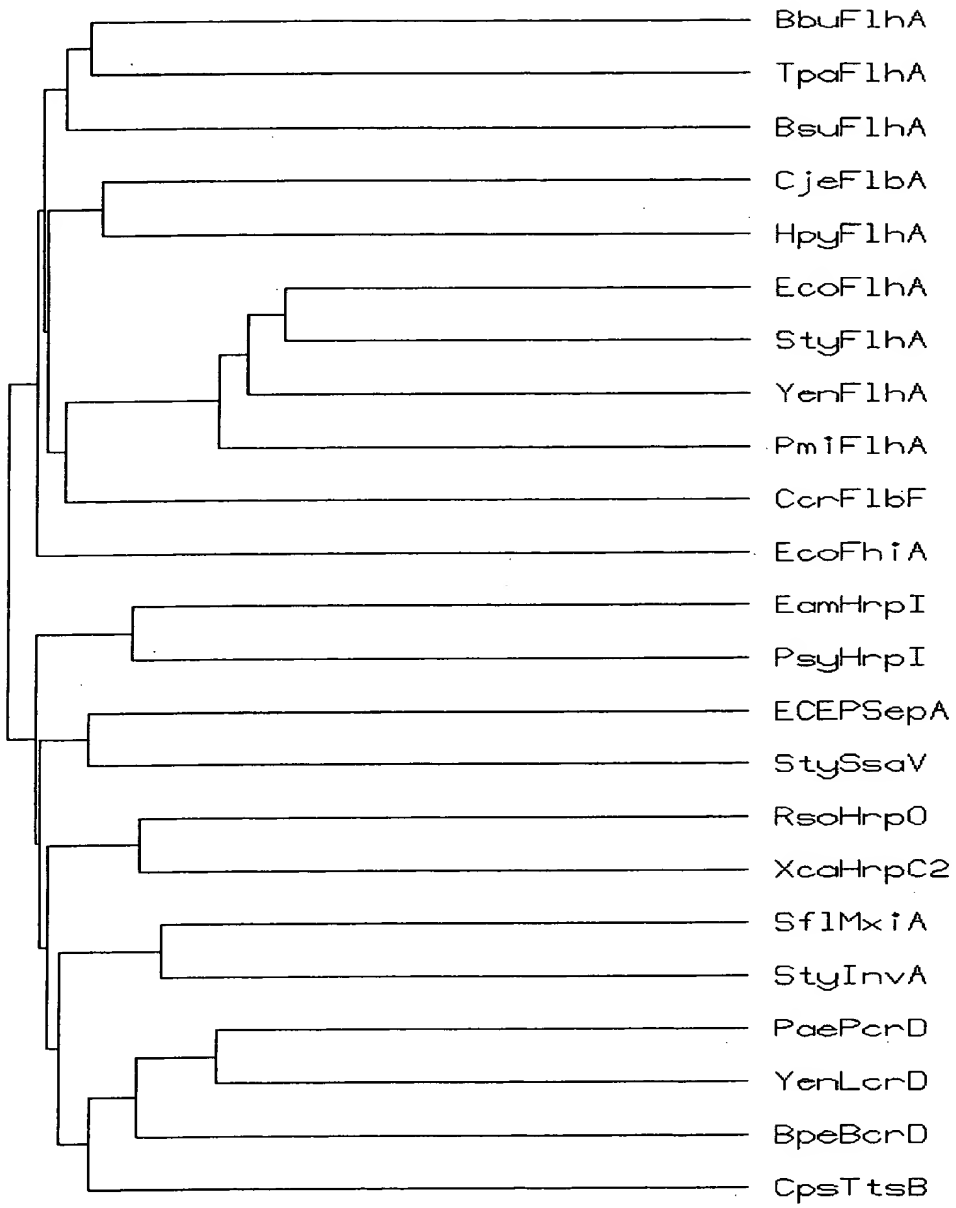
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Fig 2

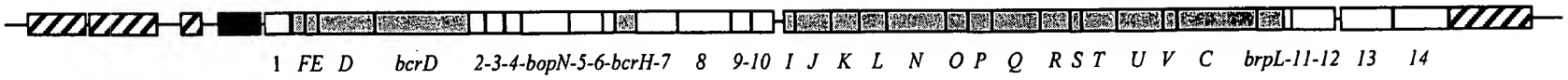
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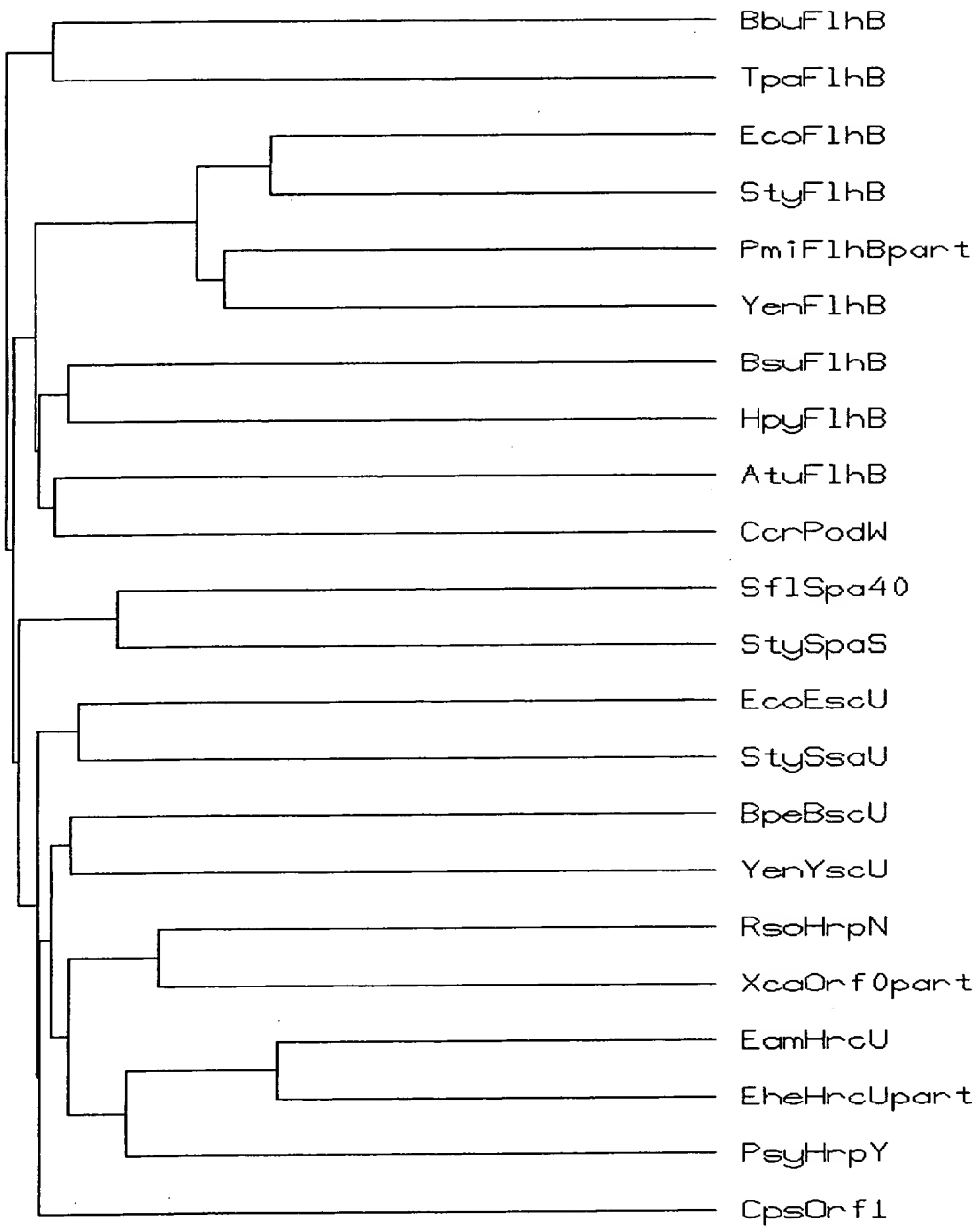
Fig 3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StySsaU
BpeBscU
YenYscU
RsoHrpN
XcaOrf0part
EamHrcU
EheHrcUpart
PsyHrpY
CpsOrf1

Fig. 4

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  51  CCTGGGTCAG GTCCTTGGGG AAAGACGTGA TGACCGTTAC CGTGCCGGCC
 101  AGGGCCGGCG CGCAGGCAGC CATGATGGCC GCTGCGAGGA CTGGGCGCAA
 151  CGCTTGCATG GGTCTCCTCC TTTCTTGAGT TGTGGCAAgA CCTTAATGCC
 201  CGTGTTCCCC CGAGTCCAAT CAGAAATTTT GATGCGCGCC ATCaACgGCG
 251  CGGCcGGCTT CAGCGCGCCG CCAGCCGCTG CGCCAGGCGC AATTGCTCTT
 301  CCAGCAGCTT CTGGATCTGC CCCTGGATGG CGAAGGCCTG GCGGTTCAGG
 351  TCCTGCAGCT GCCGGGCCTG CTGCTCGGCG GTGGCGTTGC TTTCGCGCAC
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 501  GCCCGGTCGC GCAACTGCCT GGCGTACTGG TCGAACGCGC CGGCGGAGAT
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 601  GGGTTAGTCC TTAATACGGC AGGCGCGCGG ACGAACTTAA ACATATTGCT
 651  GCGCGCTTCC GACCCCCCTT ACACTTGCGC CGCCCATCCC TTCAACTTCC
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 951  CCCAGCTACC TGGGCTCCAG CTTCGCCTTC ATCGGCGGGG TGGTGGCGGT
1001  CACCGGCTAT GTGGCGCCCG GCGCCAACGC CAATATCGGC GTGGCGCTCG
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1451  GCCGGTGTTC GAgCCGCAGG CCATGGGCCT GATCGTGCCG GTGGCCATCA
```

Fig. 5 1/23

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1551 GGACAGGACC TGGACCGCTA CGTGGGCCGC GCCTTCGTGG GCGACGGCGT

1601 GGCGACCATG GTTTCCGGCG CCGTCGGCGG CACCGGGGTG ACCACCTACG

1651 CCGAGAATAT CGGCGTGATG GCCGTGACGC GCATCTATTC CACGCTGGTG

1701 TTCGTGGTGG CGGCCGTGAT CGCGCTGGTG CTGGGGTTCT CGCCCAAGTT

1751 CGGCGCGCTG ATCCAGACCA TCCCCGGCCC CGTGCTGGGG GGCATGTCGG

1801 TCGTGGTGTT CGGCCTGATC GCCATCGCCG GCGCGCGCAT CTGGGTGGTC

1851 AACCAGGTCG ATTTCAGCGA CAACCGCAAT CTGATCGTGG CCGCCGTGAC

1901 CCTGGTGCTG GGGGCGGGCG ACTTCAGCGT CAAGCTGGGC GATTTCTCGA

1951 TGAACGGCAT CGGCACCGCC ACGTTCGGCG CCATCATCCT GTACGCCCTG

2001 CTGGGCCTGG CGCGTCGCCG CTGACGGCGC GCCAACCCGG CACcGGGGCC

2051 GCGCTCAGTG GGCCGCGGCC GGCGGcAGGG CGGCATGGCC GCCCGCCGGC

2101 CCGGCCAGCG GCGCGGCGGC GACGTCGATG ATGGCGCCGC CCTGCGTCCT

2151 GAAGACCGCC GCCGCGGCCG CCAGGTGGCG GGCCTGTTCT TCCATGGCCG

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2401 GAGCCGGCGC GCACCGTCGC CACCGAGCTC TCGATCAGGG CCTTGATCTC

2451 CTTGGCCGCC TGCGCGGCGC GCTGGGCCAG GCTGCGCACC TCGCCCGCCA

2501 CCACCGCGAA GCCCTTGCCC TGTTCGCCGG CGCGCGCCGC cTCGACCGCG

2551 GCGTTCAGCG CCAGGATATT GGTCTGGAAG GCGATGCCGT CGATCACGGT

2601 GACGATGTCG GCGATcTGGC GCGAGCTCGC GGAGATGCCG TGCATGGTCT

2651 GCACCACcTG CGCGACCGAC TCGCCGCCGC GCTGCGCCAC cTGCATGCTG

2701 ACGGCGGCCA GCTGATTGGC CTGCGCGGCA TTGTCGGCGT TCTGCTTGAC

2751 cGTGGTGGCC AGTTCcTCCA TGGTGGCCGC GGTcTCTTCC AGGGcGGCGG

2801 CCTGCTCCTC CGTGCGGCTG GACAGGTTGG CGTTGCCGgC CGAGATCTCG

2851 GCCGCGCCGA CGTTGATTTC GTCGACGCCG CGCCGCATGA CGGCGATGGT

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2951 TTTCATTGGC CGAGCGCACC TCGATGCGCG CGGTGAGGTC GCCGTCGGCG

3001 ATGCGGTCGA AATGATGGCC GGCCTCCAGC AAGGGGCGCA GCACCGCGCG

Fig. 5 2/23

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3151  CGCACTTCGT CGCTGTGCGC CTGTACCCTG GCCAGAAAGG CTTCCATGTC
3201  GCGCTGGAAT GCATGTTCGG CCTGCTGCGC GCGATGCATG GCGGCCAGGG
3251  CGGGCTCgGC CTGgCCGGCG TCGACGGCGG CGGCCAGCTC GTCCAGCACC
3301  GACTGGTAGG CGCGCCAGCG CGTCTGCAGG GTCGCGGCCA GCTCGGCCGC
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Fig. 5 3/23

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4701  GATTCCGCCC CCCTGCATAC CCATGGCCAA TGACCGACGC CGTTTCCACC
4751  ATCAGCCAGC CGCTTGCGGC CAGGCGGGGT GGCACAGCCG CAGCCGTCGC
4801  GCTGCAGGCG TCCCGGATGC GATATCCCGG GCTCCGGTCG TGGCCAGCGT
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4901  TTCCTGAACG CCGGGCCGTG CGCCGGGCGT CTGTATCGTT GTCGCGCCGC
4951  GATGCACGGA ACGTGCCGTC GGGCGCCGCA ACGCCGGGCC GCGCCGCCTA
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5101  AGCCAGGATT TCATGGCATC GGCTCGGTGT TGGGAGTTCT GGTAgGTGTG
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Fig. 5 4/23

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6201  GGATATTCCG AGAAGCAATC GGCTGGCGCC TTCCAGACTG TGCGCATACC
6251  ATTGCCCTCT TTGCCACGC ATTTCGAGCG TATGGTTTCC CTTGCGCCCG
6301  ACGCAGGCTC GCCTCGCCAT GACCGACACG GCATACCACC AACTCATCGC
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7401  TCGGGACCAT CGAAGACCAG GCGGCCGGAT TCGATGGCGG TAAGGCGATA
7451  CTGGTCCCGC AGTCCGCCCA CCAGGAGGCG GCTGCCATCG GCCAGCATCA
7501  GGTACGGTTG CGGGCCGCTC ACGACACTGC GTATCTCGAA CGGCACGTGA
7551  TCCCGCGTCG CGCGCGCGGC GGTGGCCGGC AGCCGGACGA CGTCGTAGTT
```

Fig. 5 5/23

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7651 CCGCCAATCC GCCGGGATCT GCGTCCAGGC GGTCGGCGTG CCAGCTGAGC

7701 TTGACGCCGT CGAGGCGTTC GTCGGCCAGC TGGGCCGCGA ACTGGGCCGA

7751 GACCTCGTCG GCCAGGCGTA CATCGCGACC GAGGATCGTC ATGCCCGGCA

7801 GGCGCATGCG CACCGCATGC AGCGCCGCGG CGCGTTCGTG CGCATCGCTG

7851 GCGATGCCCG AGATCGCCAG GCGGCCATTG CCGTACGGGC GCGCCATGTA

7901 GCGCACCCCG AATGTCGCCA GGACATCGCA GGCCAGGGCC CTGGCCTCGT

7951 CCTGCCTGCT TACCTGCATG GCAGGCCGTG GCGCAAGTTG CGCCAACGCC

8001 CTGGCGACCC GAGCGAATTC CGTCTCGTCG TGCACCCATC CGGTCACGGT

8051 GAGCACGCCG CCACGGCCGT AGGCCGCTTG TAATTGCTCG GTAAGGCCCA

8101 GGCTGTCGAT GAGCGCCGCG GCGCGGACCA GCGGCGCGGT GGGCGTTGGG

8151 GGCGGCGCGG CCGGCGGCGT GGCGGGTGTG GTCACGGAAA CCAGCGCCGT

8201 GGCCAGGCCG ACCAGCAGGA CGGCCGCGGC CGCGCCCAGC GCCAGCCAGG

8251 GCCGTCCTGC ACGTCGGCGC GGCATGAGGG CAGCGACGGA CGGCGGGCTT

8301 GTCGAGCCAG GGACGTCGTG CAAGGCTGTG TCGCTGCCGT CCGGGCCGCA

8351 CGGCTCCGGC GGCGCGGGCC ACGGCGCGGA AGGGGCGGCC ACGGTGATCC

8401 AGGCGGCTCC CAGCTCTACG GGTTCGTTGA AGGCCGCGGG CGGACACGGC

8451 GCCTGGGCGT CCAGGCCGGG CGTCACGGCG CCGGCCAACC GCCAGCCGGA

8501 CTGGTCGATC TCCAGCCATC CCGCCACTTC GGGCATGTCC TCGCCGGTCA

8551 GGACGATATC GCAATGCGGA TTGGCGCCCA CGCGCGCGCC ATGCACGGCC

8601 GGGCAGCGCG CCATGCACTG TGCGCCTGAA AGCACGCGGA ATTCCAGCGC

8651 CGTCGTCATA GATCCACCCT GCCCAGGGGC TGTACATTGA TCTCCGGCGT

8701 CAGTTCCTGG TAGGACAGCA CCGGCAGGGC GTAGAGATCG GCTTCTATCA

8751 TCTTGCGCGT GTAGCGCCGG ATGTCCATCG ACGTCAGCAA GACGGGACGG

8801 CTCGCGCCGG CGGCCAGATC GCCGACACAT TGACGGATGT GCTCGACCAG

8851 TCGGCGTGTC GTGTCCGGAT CGAGGGCGAG ATAACTGCCG GCGGCGGTCT

8901 GCCGGATGGC GGCGCGCACG GTTTCCTCGA CCTTGGGGGC CAGCAGGTAG

8951 GCGGGCAGGA TATTGTGGCC GCTGGTGTAC TTGTGGCTGA TATAGCGCTT

9001 GAGTGCGATT CGGACATACT CCGTAAGCAG GACGGTATCC TTTTCCTTCT

9051 GGCCCCATTC GACCAGCGCT TCCAGGACGG CGCGCAGGTT GCGTATCGAC

9101 ACTTCTTCGG AAACAAGGCG CTGCAGGATT TCGGCAATCT TCTGCACCGG

Fig. 5 6/23

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 9151  CATGACGCGC AGGCACTCCT TGACCAGATC GGGAAATCGT TCTTCCATGG
 9201  CCGAAAGCAG AAACCGGGTT TCCTGGATGC CGATGAAATC GGCTGAATAT
 9251  TTTTTCAATA CATATGCCAA GTGCCAAGTC AGGATCTGGC TGATACCCAG
 9301  GTAAGGAATA CCTGCATCGC GCAAGGCGCC GGTCAGACTG GCCGCAACCC
 9351  AGATCGTGGG CGTATCGGGC AGAAAGGCCG CGCCCGTTTC GTATGCGATC
 9401  CGCAGGGCCT GCAGGTTCTG CTCGGTGTCC CGCACCAGCA CGGCATCGTC
 9451  GCGCAACATT CCTTGCGCCA CCGGGATCTC CGACAGCACG ATGGTGTAGG
 9501  TATTGGCGGC CAGCGCTTCG GTGAAGCGCA ACTGGATGCC GGGAAACGGC
 9551  ACGCCCAGGT CGAAATAGAG CGCCCGCCGG ATCTGCAGCA GATCGTCGGT
 9601  GAGGGTGGCC GGCTCGAACC GGGGCTGCAG CCGCGCGGCT ACGTCGATGA
 9651  TCAGCGGGAC GGTGGGGGCG AATTCCGCCT GCCCATCCGC CGGCGCGCGG
 9701  GTGCGGGGCT GGCCGTCGGC AGCCATGCCG GCGAGCGCGG GCTCGGCGCC
 9751  TTCGGGCGGA CGCTGGGATG CGCGCAGCAG TACGAAACCG ATGGTGCCCA
 9801  CCGCGGCGGC CAGGGCGAAG AAGACCAGCG TGGGCATGCC GGGAATGAGG
 9851  CCCAGGCCTG CCGAGATCGC GCCGGCAATG ACCAGGGCGC GAGGCTGCGC
 9901  CAGCACTTGT GCGCCGATGT CGGTGCCTAC GTTGGAGGGG CCATCCCCGG
 9951  TCTGCACCCG CGTCACGATG ATTCCGGCGC AGATGGCGAT GAACAGCGCC
10001  GGGATCTGCG CGATGAGCCC GTCGCCTATG GTCAGGATGG CATATGTCTG
10051  CACGGCCTCG CCGGCGCTCA GGCCGCGCTG CAGCACGCCG ACCAGCATGC
10101  CGCCAAGCAG GTTGACGGCA ACGATGATCA GGCCGGCGAT GGCATCGCCC
10151  TTGACGAACT TCATCGCGCC GTCCATGGCG CCATACAGTT GGCTTTCCTT
10201  CTCGACCGTA CGGCGTCGGC GTCGGGCTTC GTCCATGTCT ATGGTGCCCG
10251  CGCGCAAGTC CGCGTCGATG GACATCTGCT TGCCGGGCAT GGCGTCCAgC
10301  GAGAAGCGCG CGGCGACTTC GGCCACCCGC TCCGCGCCTT TGGTGATGAC
10351  CACGAACTGC ACGATCGTGA GGATGAGGAA AACCACCAgG CCGACGATCA
10401  GGTTGCCGCC CACCACGAAG TTGCCGAAGG TCTCGATGAT GTGGCCGGCA
10451  TCGCCTTGCA GCAGGATCAG CCGCGTGGTC GCGATGGAGA TGCCCAGCCG
10501  GAACAGCGTG GTGACCAGCA GGACCGAAGG GAACGAGGAA AACGCCAGGG
10551  GCGAAGGCAg GTACATCGCG ACCATCAgCA GGACTGCCGA CAGCGTCATG
10601  TTCGCACCGA TCAGCACGTC GACCAGCGTT GTGGGCAACG GCAGGATCAT
```

Fig. 5 7/23

10651 CATGAAGACG ATCGCCACGA TGAGCACGGC CAGTACGATG TCGTTGCGGC

10701 TGGTGGCCAG CGCCACCGCG CGTTGCAGGC GGCGAATGGA TTTCTTGCTC

10751 GTCATGGCGG GGTCGCGCTC GCGCAAGACG CCGCCCGTAG CGCCATATAG

10801 TCACGCATGG CCTGGCGGGC GTCGTCGGGT CGGTTCAGCG CCTGCATGGC

10851 CTGCGCCCGG ACCAGATGGC CGGCGGCATC GGGTGTGGCG CGCAGTGCGC

10901 GCTTGTCCAG CGTGACCAAG GCCATGCGCG GTTCGCCCTG GTGCAGATAG

10951 CCCAGCGCCA GGGCCAGCAG GGACTGGCTG TCGATCGCGT CCAGGGCATC

11001 CAGGGCCGCC AGCAGGGCAA CCGTCTTGCT CCATTGGCGC TGCAACTGGT

11051 AGTGGTGCGC AAGCAATTGC AGAAGTTCGC GTACCTGAAG GCTGGGCGAG

11101 GGTAGGGTAT GCGGCATCAT CCCTGGTAGA GCGCGCTGCG ATACATGGCA

11151 GCCAGGTCGC GCAGCTTTCC GGCCTCGTTG AGTACGTGCA AGGCGCGGCT

11201 TAAGGCTGGC GCGGTGTTGC CGGCATGCAG TTCCATGGCC CGGGACAGTT

11251 CGTCGCGCGC CTGGGCCAGG GCGCGCTCGA ACTGCGCGGG CTGGAATAAT

11301 GCGCCATCCG TCAGGCGAGG CCGCGCCGAC TCGTCGAGCA TCGCGCTCAG

11351 GTCCGGGCGT ACGAGCAGGG CTTTCAGATG ATCGACCGCG CCGGTGGCGG

11401 GCGGTTCGAG CCAGCGCTCG GGTGGCAGGG TGGGGGCGGG CTCGCAGCGG

11451 GGACCGCGCA CGATGTGGTC GACGCCGCGC TCCAGGCCGA GGTGCATGGC

11501 ATGCATGCCG GGTACGGCGC TGGACATGGC GTCACGCCTC CAGGCGGTCG

11551 AGCCAGTTCG TCAGGCCCAG CACCGCCAGG CGCAACGCCG CTGCGTCGAG

11601 CGAGCGTTCG GGCAGTCGTG TCTGCGCGAC GATCCAGTCC TCGCTGCCCT

11651 CCGACCAGAG TGACGTCTGG ATGGATGCGG CGCTTCCGCG CTGCCCATGG

11701 GCCCGTTTCC ATGCCGCCAG CAACACGGAG GCGGCGTCGC GCTCGACCCG

11751 CTGGGCCAGG TGGACCAGGG CCGCGCCGGC GACGCATTCG ACGCCCAGGC

11801 GGCGCCCGTT GGACAGCGCC AGCGACGCCG ATCCGGACGG CCCGAATGCC

11851 AGGCCCTCGA TGCCGATATC CTGGCCGAAC TGATGCAGCG CCCTATCGGC

11901 AGTATTCATG CGTTCTCCAT TGCTATCGCG TTGTCCAGCG CGTCCTGCGC

11951 GGCCGCCAGG ACGGTGGCGC GCACGTCCAT GTCGGCGTAG ATCTGCGTGG

12001 GCAGGTCTTT GAGAATCTGG CGTACGCCAC CGAGGAATGC GATGCGCTCG

12051 GAGAGGGCAT TCGCACCGTG GCGCTCGGCC AGCTTCTCGA AGCGCGCGGG

12101 CGCAATCCAT TTGTCCTCGC TGATTCCCAC AAGATCGCGC ATCAGGCCCT

12151 GGGCGTCCGC ACACTCCTGC GAGCCTGCGT TGCCCAACCG TTGTTTCAGG

Fig. 5 8/23

```
12201  GCATTGCATT CCTCCAGTAC CGTGGCGGCC ACCTCGACTT GATAGAGATC
12251  GCTCGCCAAC ACTTGCAGCC TGACGCCGTC CGTCGACGGT GTCGCCGCGG
12301  CCAGGTCGTG TCCCAGCGCC TGAATCAGCG CGCCCAGCGC GCCGTGGATG
12351  TCGTCGTTCC CATAGCGTTC CAGCACCAGG TCCAGCGTGC GCGCCAACGA
12401  CAGCTGGCCC AGGGCGATGT CGCGGTACGC GTGCTGGAAG CCGGCCAGCT
12451  CGTCAGCGGA ACGCGCGAAT GCGCCGGCCG TGGGCAGGGT GTTGATGCCG
12501  GCGCGGATTT CGGGGCCATG GGCGAGCTCC AGGTCGGCCA ATGCATCGCG
12551  CAGGGCTTCG AGCGCGTGCG GCGCGGCGTC CTCGTGCTCG CCGCGCTGCA
12601  GCGCGTGCTG CAGCGCGAGG TATTGCTGCG TGACACCGGG AAACGCTTGC
12651  GCGGCCAGCT GCATGGGGGC GCCCCGGCCG CGCAGCAGCT CGGCGGTCAG
12701  GGCTTCCAGT TTTGCCTGCG CGTCGGGGTC GTGGGTGTGG GAAAACAGTT
12751  CCGCAAGCTG CGCCGCGTCC AGCCAGAGCA TCGGACGTTC GGCCGTGACC
12801  TTGCGTTCGG AGTGATGCTT TTCCTCGGCA GCCTGCGCCA TGTGCAGGCT
12851  GAGCTCCTCG GCCGCGTCCG CCAGCGATAT GCCGGTGGGC GCCGGTGCGA
12901  TGCGCTGGCC TTGCAGCCAG CCGGAGGAGG TGTTGGCCGA GGCGTCGTGG
12951  CGCCCCTGCA TGGCGGCGTG GAAGGGATTG GGGGCGGCAT CGATACGAGT
13001  CATGGGGAGT CCTCGGAGAA GGAACCATTT GCCTACTGGT GCAGTGAGTG
13051  TCGCGCGCGC GGTCATGGTT CCCGGAAACG GGCGCGATAT TGGGCAATTC
13101  GCAGCCTGGA ACTTGCCGCG GGCGCAGGGT TACTCAGCAT GCGTCTTTCA
13151  ACTCGAAGGA GCTCTCATGA GCATTGATCT CGGAGTTTCA CTCACGTCGC
13201  AGGCCGGCGG CCTGCAAGGC ATCGACCTCA AGAGCATGGA TATCCAGACT
13251  CTCATGGTGT ATGTGCAGGG TCGTCGCGCC GAACTCCTCA CGGCTCAAAT
13301  GCAGACCCAG GCCGAAGTGG TGCAGAAGGC CAATGAACGC ATGGCGCAGC
13351  TCAACGAGGT CCTGTCCGCG CTGTCCCGGG CCAAGGCCGA GTTTCCGCCC
13401  AATCCGAAGC CGGGCGACAC CATCCCGGGC TGGGACAGCC AGAAGATCAG
13451  CCGGATCGAG GTTCCTCTCA ATGATGCGCT GCGTGCCGCC GGCCTGACGG
13501  GCATGTTCGA AGCGCGCGAT GGCCGGGTGA CCGGCCCCGA CGGCCGGGGT
13551  ACGCAGGTCG TGAACGGCAC GGGCGTCATG GCCGGTTCCA CGACCTATAA
13601  GGAACTCGAA AGTGCCTACA CCACCGTAAA GGGGATGCTG GATACGGCGT
13651  CCAATACGCA ACAGATGGAC ATGATCAGGC TGCAGGCCGC CAGCAACAAG
```

13701 CGCAACGAGG CTTTCGAGGT CATGACCAAC ACCGAGAAGC GGCGCAGCGA

13751 CTTGAACAGC TCCATCACCA GCAACATGCG CTAAGCGCTG CACAAGGAGT

13801 ATTCCATGCA GGAGCAAGGC ATCCAATCCA TCATGCGCGC CGCGGAAGAG

13851 CTGGTCGAGC AGACCCGCCA GGCGTTGTAC AGCGTCGACG AGATCTACGC

13901 CCACGTTGGC GTCGACCCCG CTCGCCTGCG CAATCTGGCG GTCGAGCAGG

13951 CCAGGATAGA GGCCGAGGCC CAGGCGGCGT TCCGTGATGA CCTCGCGGAC

14001 ATCGAGCGCG AGGCGGCGCG CGTCAAGGCG GCCTGCACCG ATGCGCCGCA

14051 GGCCCGCAGG GTGCTTCACA ACCACGTCTG AGCGCGGAGG CCTTCCATGC

14101 CAAAGTCAGC CGACCAGGGC GGCTCCCCGG CGTCAGCTTC GCATGAGGCG

14151 TTGCGCCATA TTCTCGACGC AGGCGCTTCG ATGGGGGGCT TGCAGGGGTT

14201 GGACGAGGCG CAGCAGCAGG CGTTGTACGC GATCGGTCAT GGCGCCTACG

14251 AACAGGGGCG CTATGCCGAC GCGTTGAAAA TGTTCTGCCT GCTGGTCGCG

14301 TGCGATCCGC TGGAAGCCCG TTATCTGCTG GCCCTGGGCG CCGCGGCCCA

14351 GGAGCTGGGG CTGTACGAGC ATGCCTTGCA GCAATACGCG GCCGCGGCGG

14401 CTTTGCAGTT GGACTCCCCC AGGCCCCTGT TGCATGGCGC CGAGTGCCTG

14451 TATGCGTTGG GTCGTCGCCG CGACGCCCTG GATACGCTCG ACATGGTGCT

14501 TGAGTTGTGC GGCTCGCCGG AGCGTGCGGC CCTGCGCGAA CGGGCCGAGT

14551 TGCTGCGCAG GAGCTATGCA CGTGCCGACT GAAACGGCGC CATGTCCGCC

14601 GTCAAGATTT CAATTCGAGG AGGTTCGATA TGTCTGTTTC TCCGACTTCG

14651 CCCGGCTCTT TCGGGGCCGG CCCTGTCTTT GACTCCGAAT TGCAGGCCCC

14701 GGCCCCGTCG GCGCAGCGTC GCGGCGGTGC GGCGCCTGTG CCGCCGCCCG

14751 TCGATCGGCG CGGCGTCGAG CCGGGAGATC CCACGCTGGG CATGCTGCCC

14801 GCGCCAGATT TGCTCGCGGG GGGCGCCGTC AGCCGCACCC GCGCGGCGCT

14851 CGACGATCTG GACGCCGCAC GGCTCGGTGA AGACATCTAC GCCTTGATGG

14901 CGGTGTTGCA ACAGGCCAGT CAGCAGATGC GGGACGCCGC CCGTATCGCT

14951 CGTGATGCCG AGGCTACGCG GCAAACGCAG GCTCTCGGCG ATGCGGCCAG

15001 CCAGATGCGC CAGGCGGCGA GCGAGCGCAT GGCCGGAGCG ATCGTGGCGG

15051 GCGCCATGCA GATAGCGGGT GGTTTCGTGC AGCTGGGGGC GGGCCTGGCA

15101 GCGGGTTTGC AGGCCATGGG TGGCGCTGCT GCGCAAGCCA AGGGCGCCGC

15151 ATTCTCCGAG CAGGCCTCGA CAAGCCGCAA GGTGGCGGCC GGCTTGCACG

15201 ATGCCCCCGA GCTGCAGGCA ACGGTGCAGG CCCGCGCAAC CCAGCTCGAA

15251 GCGCAAGCGG CCTCGTTTGG TGCGGACGCG GCTCGTTCGT CGGCAAAGTC

15301 GCAGCGCGTA TCGAGCGTTG CCCAGGCCGG CGCCGCAGCG GCCGGCGGTA

15351 TCGGCGGCCT GACCAGCGCC GCCCAGGAAC GCCGCGCCGC CGAGCACGAG

15401 GCCAGGCGCG CGGAGCTGGA CGTCGAAGCG AAGGTGCATG AAACGGCCTC

15451 GCGGCGGGCC GACGAAGCCA TGCAGCAGAT GCTCGACATC ATCCGCGGCA

15501 TCAGGGAAAA GCTGGCCGGG ATGGAGCAGT CCCGCAGCGA GACCGCCCGT

15551 AGCGTGGCCC GCAATATCTG AGTGTCCGGC TCCAACCTTC AATCTTGAGG

15601 ATGACCGTCA TGAGTACGAC CATATCCACA GCCCCGAGCG GCGCCGCGCT

15651 TGCGCCGTCT CGCATAGATA TGCGGGCGCC GGAGCCCGGG AGTGCCGGCG

15701 AAGGCGCCGG TATCCTGGCG CCGGTGACGA CGCTGGCTCT GGCGGCGGGC

15751 CGGCCGGCTT TGCCAGCGTC ACCGTCGCTG CGCACCGCGC CCGTCCTGGA

15801 TCCGCCAGTG CGCGATCTCA GCCCCGCCGA CTTGGCCGAC CTGCTGCGCG

15851 TCTTGCGATC CAGGGCGGTG GACGGGCAGT TGGCCACGGC GCGCGAGAAC

15901 CTGCAGGATG CGCAAGTCAA GGCGAAGCAG AACACCCAGG CCCAGCTCGA

15951 CAAGCTGGAC GCATGGTTTC GGAAGGCTGA GGACGCCGAG AGCAAGGGCT

16001 GGCTGAGCAA GGTGTTCGGC TGGATCGGGA AGGTGCTGGC GGTCGTGGCA

16051 TCGGCCCTGG CTGTGGGCTT TGCTGCCGTC GCCAGCGTGG TCACCGGCGC

16101 GGCGGCCACG CCCATGCTGG TGCTCAGCGG CATGGCATTG GTCAGCGCCG

16151 TGACATCGCT GGCCGACCAG ATATCGCGAG AGGCGGGAGG GCCGCCTATC

16201 AGCCTGGGCG GGTTTCTCTC CGGGCTGGCC GGACGTCTGC TGACAGCGTT

16251 GGGGGTGGAT CAGTCGCAGG CCGACCAAAT TGCCAAGATC GTCGCCGGCC

16301 TGGCCGTGCC CGCCGTCTTG CTGATCGAAC CCCAGATGCT GGGCGAAATG

16351 GCCGAAGGCG TGGCCAGGCT GGCGGGCGCC GGCGATGCCA CCGCGGGATA

16401 CATAGCCATG GCGATGTCCA TCGTGGCGGC GATCGCGGTC GCCGCGATCA

16451 ATGCCGCCGG TACGGCCGGC GCGGGCAGCG CCTCGGCGAT CAGGGGTGCC

16501 TGGGATCGGG CCGCCGCGGT AGCCACCCAG GTCCTTCAGG GGGGTACGGC

16551 AGTGGCGCAA GGCGGCGTCG GCGTGTCGAT GGCAGTCGAT CGCAAACAGG

16601 CCGATCTCCT GGTCGCCGAC AAGGCGGATC TGGCGGCGAG CCTGACAAAA

16651 CTGCGGGCGG CCATGGAGCG TGAGGCGGAC GATATCAAGA AGATCCTGGC

16701 TCAATTCGAC GCGGCCTATC ACATGATCGC GCAGATGATC AGCGACATGG

Fig. 5 11/23

16751 CGAGCACGCA CAGCCAGGTC AGCGCCAACC TCGGACGGCG CCAGGCGGTG

16801 TAGCGCCGGG CGCTCAAGGA ATTTTCATGA CTGTTCATGA CGACGCGGCC

16851 GCGGCGCTGC GCGCCCGGCT GGATGCGTTG CCGGGCAGCC GGCGCCTGAC

16901 AGCCGAGCAA TTGGAAGTGA TTTACGCGAT GGCGTATGCG CACGTCGCCA

16951 GGTGCGAGTA CGGCAAGGCG CTGCCCATTT TCGCCTTCCT CGCGCAGTAC

17001 GGCCCCACGC GCAAGCACTA CTGGGCCGGC CTGGCGCTAT GCCTGCAGAA

17051 GACCGACCGT CCCGACGAGG CGCGCAATAT CTATGCGTTG ATCCTCACGC

17101 TCTATCCAGA TTCCGCGGAT GCCGTGTTGC GCACGGCCGA GTGCGAGCTG

17151 GCGTTGGGTG AGAACGAACG GGCGCAGGCG GCCCTGTTCG GCGCAATCGC

17201 CATCGATGCA GAAAGTGGGC AGCCAGGTCC GGTCTCGCAC CGTGCGCGCG

17251 CTTTGCTCGA TCTTATTTCA GTTTCACATC CGGAGTAACT CCATGCACTC

17301 AGACTCAGGT TCAGATTCAG GCTCAGACTC AGGCTCaGGC TCACCCATGG

17351 TCTCGTCGAT ACATCCATCG GAACCGATAC AGCCGATGGA GCATGTGCTC

17401 GAGGAGGCCG ACGCCCGCCT GCTTACCGAA GTGGGTTTTC TGGCGGCGGC

17451 CGTCAGCGAT CTGACGCGCG CGGACGCCAT TTTCAATGCA TTGCAACGTG

17501 TACGGCCGGG CCGGACGCAT CCCTGCATCG GCCTGGCGGT CGCCCGCATG

17551 AACGCCGGGC TGCCCGACGA AGCCGCCGAG ATCCTGGCGA ATTTCCAGCC

17601 GGCACAGCCG GAGGACCGCT CGGAACTGGA CGCCTGGTGC GGGTTCGCTC

17651 TGTTGCTGGC TGGCCGCTCG GACGAGGCGC GCCGCATGCT GCAGCGAGCC

17701 ATCGATGCGG GTGGCGAGGC GGCAAGGCTG GCGCAGGTCG TGTTGGACAG

17751 CGGACCCGCC ATGATGCGGC CCGCGCCGTT GCAGTCCGAG CCATTACCTG

17801 GAGCTCCTGG ATGAATTTGG ATCTGACGGC GATCAACGCC GTGCAGGAAC

17851 GGCTGCTCGC TGGATCATTC GACATGCCGC GATCTCCCGC GATGGCGGAT

17901 CAGGCGCGCT TTGAATTGGC GCTGGGCGAG ATGCCCGGCG CATCGGCCCC

17951 GAACGGGGCG ATCGCCCTGG CGCCGGTCGC GCTCGACGAG CCGCTGGGCC

18001 GTCGCATTCT TGGACAGTTG CGCGGCGGCC TGGCCGATGT GGCAGGAAAA

18051 TGGCGGGCGG TGCAGACGGG CTTGGCCGAG GTGAGCCAGG CGCCTACCGT

18101 GGTGGGTATG CTCGATCTGC AGGCCAGGTT GCTACAGGCA TCCGTGGAGT

18151 ACGAGTTGGT GGGCAAGGCA ATAGGGCGCG CCACCCAAAA CGTCGATACG

18201 CTGGCGAGAA TGTCATGAAC GCCATCGGGG CGATCCAACG GTATCGGCGC

18251 GGCGCGGGAT GGGCGGCCCT GGTGCTCGCC CTGGCGCTGC TGGCCGGCTG

18301 CGGTGCCCGC GTCGAGCTGT TGGGCGCGGC GCCCGAGAAC GAAGCCAACG

18351 AAGTATTGGC GGCGCTGCTC GAGGCAGGCA TCGCTGCGCA GAAGCAGTCC

18401 GGCAAGGCCG GCTACGCGGT TTCGGTGCCG GCCGAGGCGG TGGCCCGGTC

18451 GCTGGAGATC CTGCGCGCAA GCGGCCTGCC CCGCGAGCAG TTCGACGGAA

18501 TGGGACGCAT ATTCCGCAAG GAAGGCCTGG TTTCATCTCC GCTCGAAGAG

18551 CGCGCCCGCT ACATTTATGC GCTGTCTCAG GAATTGGCCG ACACCCTGTC

18601 GCAGATCGAC GGCGTGCTCA GCGCCCGCGT GCACGTGGTG CTTCCCGAAC

18651 GCGGCGCGGT CGGCGAGCCG GCCACCCCTT CGACGGCAGG GGTGTTTCTC

18701 AAGTACCGCG ACGGACAGAG CCTCGACGCG CTCGTGCCCG AGATCCGCAA

18751 GCTGGTCACG CATGCCATCC CGGGCCTGGC CGAGGACCGT GTATCGGTTG

18801 CCCTGGTGGT GGCCCAGCCC GTTCAGGCCG CACCCGCGCC GGTCGCGTGG

18851 CGCCGCGTGC TTGGCGTACA GGTCGCGGAC GGATCGGTCC TGAGATTTTC

18901 GCTGTTGCTG CTGTTGTTGC CGGTGCTGTG CCTGATAGTG GCGGGGgCCG

18951 CGCTCTACGT CTGGCGCACG CGCTGGTCCC GCGGCGAAGG GCGCGGCGGC

19001 GCTGGCGCCG GCGCCACGGA AGGAGCCGGG CATGACTGAG GCGAGCGTGC

19051 TGCTTTCCGA GCGGCTCATG ATATTCAATC TCCTGCCCAG CCTGACCCTG

19101 CATGCCAGTC GCCACGACGA GATGTTTCCA GCCGATTGGG TGCGCGCGTT

19151 GTGCAATGCC GACGCGGCGT TGGCCAACGC GTGGCATCGC CATTGGTCGC

19201 GCTGGATCTT GTGCGAATTG GGCCTGCTGA ACCAGCCGGT CCTGAGCCTC

19251 GATCCGCCGC AGTTGAAGGT CGCGCTATTG TCCACGGACG CCTTGCGGAC

19301 CTGCGCCGCC CATGCGGGAG CGCTGCTGTG CGCGCCGCGC CTGCGACGCG

19351 CGATAGACGG CGCCGAGGTC CGTACCTTGC ATGCCGCGCT CGGGCGCGAT

19401 GTGATGAATT TCGCCGTGTC TTCCGCGGCG CGGGCCCTGC ATGACGGGCT

19451 CGCCGCCAGT TCGGACTGGA CCCTGGCCGC CACGGTCCAG GCGGCGCAGA

19501 AACTGGGcTG GGCCCTGCTG CGCGACGCCG TGCAGGGCGC CGCCGACGAG

19551 ATAGCGCTGC GTTGCGCGCT GAAGTTGCCG CGCGACCTTG ATCCCGCGCC

19601 CGTCCTGCCG CCCGAGGCGG CGCTTGCGCT GGTGCTGTCC ATGCTCGAAA

19651 TCCTGGATGC AGAATGGCTT TCCTCGTTCC CCGCCCAAGC CTGATCCAGG

19701 CGGTACGGCC CGGCCGTGCG GATCCCGCGA CCGACGTCTT GCGCGCTGAA

19751 GACTACGCCG AGCTGCTCAG CGCCGCGCAG ATCGTTGCCC AGGCACACCG

19801 GCGGGCCGGC GAAATCGTGG CCGAGGCGCG AGAGGAGTTC GAGCGCGAGC

19851 GCAGGCGAGG CTATGAGGAG GGGCGCCGCG AAGCGCTTAC GGATCAGGCG

19901 GAGAAGATGA TAGAAACCGT AAGCCGCACG ATCGACTACT TCGCGGGTAT

19951 CGAGAACGAG ATGATCGAAC TGGTCATGAG TGCGGTCCGC AAGATCGTCG

20001 ACGGTTACGA CGACCGCGAG CGCACCGTGA TCGCCGTGCG CAACGCATTG

20051 GCGGTCGTGC GCAATCAGCG CCAGATGACC TTGCGCCTGC ACCCAGACGA

20101 GGTGGATGTG CTCCGGGAAG GCATGAACCA GCTTCTGGCG GCCTATCCGG

20151 GCGTGGGCTA CCTGGACCTG CTGCCCGACG CCAGGCTGGC GCCGGGAGCC

20201 TGCATACTGG AGAGCGAGAT AGGCATGGTC GAGGCCAGCC TCGAGGACCA

20251 GCTGTGCGCC TTGCGGGCGG CCTTCGAACG TACATTCGGC CgGCGCGGAT

20301 AGGGGCATGC GTCaGTACCa CTACATCACG GAgATGATGC GGGTGGCCCT

20351 GCAGGATCTG TCCACGCTGC GGATAAAGGG CCGGGTGGTG CAAGTGGTGG

20401 GAACGATCAT CAAGGCCGtC GTTCCGATGG TCAAGATCGG CGAAGTGTGC

20451 CTGCTGCGCA ATCCCGGCGA GGACTTCGAG ATGCACGGCG AAGTGGTGGG

20501 CTTTGTCCGC GACGCCGCCT TGCTCACGCC TATCGGCGAC ATGTACGGGA

20551 TTTCCTCGGC GACCGAGGTG ATACCGACCG GACGCACGCA TATGGTCCCC

20601 GTCGGTCCGG GCTTGCTGGG ACGCGTGCTG GACGGGCTGG GACGTCCGCT

20651 GGACGCCGCC GAGTCAGGGC CGCTGCATGC CCACAAGTTC TATCCGGTCT

20701 TCGCCGATGC GCCAGACCCG CTGACGCGTC GCATCATCCA TGCTCCGCTG

20751 GAGCTGGGGG TGCGCGTACT GGACGGTTTG CTTACATGCG GGGAAGGCCA

20801 GCGTCTGGGA ATTTTCGCAG CCGCCGGCGG CGGCAAGTCG ACCCTGCTGG

20851 GCATGCTGGT CAAGGGCGCC GCGGTCGACG TGACGGTGGT GGCGCTGATC

20901 GGCGAGCGTG GGCGGGAAGT TCGCGAGTTC CTTGAGCACG AACTCGGTCC

20951 GGAGGGCAGA CGCAAGAGCG TGATCGTCTG CGCGACCAGC GACAAGTCCT

21001 CGATGGAGCG TGCCAAGGCG GCGTACGTCG CAACCGCCAT CGCCGAATAC

21051 TTCCGCGATC AAGGGCAGCG TGTACTTTTT CTGATGGACT CGGTCACCCG

21101 CTTTGCGCGA GCCCAGCGTG AAATCGGCTT GGCGGCAGGC GAGCCGCCGA

21151 CGCGGCGCGG CTATCCACCG TCGGTGTTCG CCACCTTGCC CAAACTGATG

21201 GAGCGCGCCG GCATGAACCA GACGGGTTCG ATCACGGCGC TGTATACGGT

21251 GCTGGTCGAG GGGGACGACA TGAACGAACC GGTGGCCGAC GAGACGCGTT

21301 CGATACTGGA CGGCCACATC GTGCTCTCGC GCAAGCTGGG AGCGGCGAAT

Fig. 5 14/23

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21351  CACTATCCTG CCGTCGACGT GCTGGCCTCG GCCAGCCGGG TCATGAATGC
21401  CGTGGTGTCG CCGCGTCACA AGTACCTGGC CGGACGTATG CGCGAACTGA
21451  TGGCCAAGTA CCAGGATGTC GAGCTGTTGG TGAAAATCGG CGAGTACAAG
21501  CAGGGCGCCG ATGCGTCGAC CGATGAGGCG ATACAGAAGA TCGGACAGAT
21551  CAATGCGTTT CTCAGACAAC TAACCGACGA ACGCGAAGCA TTCGAGGATA
21601  CCGTACTGCG CATGGCTGAA ATCATCGGAC CCGAATCCTA ATGGACCTGG
21651  AAAGCCTGCT TGCCATCAAG CATTTTCGCG CCGACCAAGC CCAGCTTGCG
21701  CTGAAACGCC AACAGCAGGC CTGCGCGGTT GCTGCCGCGG CGCAGCGTCA
21751  GGCGCAAGGC CGCCTCGACG ATTGTCGCCT GTGGGCCGGA CAGCTCGAAA
21801  ACCGTCTATA TGCCGAGCTG TGCCGGCGCA TCGTCAAGAC ACGCGACATC
21851  GACGAGGTGC TGCAACGAGT GGGCCACGCC CGCGACCGCC AGGCCAGCCT
21901  GGCGCTGCAG CTCGACGACG CCGTGCGCCG TCACGAACAT GAAATCCAGC
21951  TGCTCGCGCA GCAGCGCGAG CAGCACCGGG AGTGCTTCCA GGCGCAGCAA
22001  CGGATCGCCG AGTTGGTGCG CCTGCAGCAG GTCGAGGCGG CGGCCTTGCG
22051  CGAGAGCCAG GAAGATCGCG AAATTCAGGA AGCCATCGAA TTGTCGGCGC
22101  GTGGGCGCGA CGATGCATCG CGAGCCGGCG ACGGCCTGGC GCGGCTATGA
22151  ACCAGCCAGA CGGGCTGGGT TCGCCCATGG CCGGCGGCGG GCAGCGCATG
22201  GGCGTGGCGC GCACGCCGTA TGCGCGTCAG CCGGATCGGG ATGCGCAGCG
22251  TGCCTTCGAG CGGGAAATGG AACAGGAGAA AGCGAAGGAA GAACTGCCCG
22301  GGCCGCAACG CCTGGCGCCG GGTCCGGCCT GCGTCGGCTG GCTGGCGTCG
22351  ATGGAACCTG CCGCCGGCCG TCCACCGGCC AGTCTGGCCC AGGCGCTGGC
22401  AAGCGTGGCT GCGGGGCTGG CGGTAGGCGA CGTGCTGGAG GGGTATCGCG
22451  AAGCCCGTAT CGTTGTGGAC GATACGCTGC TACCCGACAC CACCTTGTCG
22501  GTACGGGAGG ACGGCGGCTG GATCGTGGTG GCTTTCGCAT GCCGACAACG
22551  GGACGCTTGC GAGCGCCTGC ACGCGTGCGC CGACCGGTTG GCCATGGAGC
22601  TCGCGCTGGA GCTGGCGCGC GACGTCGAGG TTGCGGTGGC ATGCGACGGC
22651  GAGCCGCACG AGCGGGTGGC GCGCGCGCAG CGGCCGTGGC GATGAATCGA
22701  GTGGCCGGCG GGGCGGCGGC GCAGGCCGCT GGCATGGTGG ATCTCGCGGT
22751  TCCGCGGTTG AGCGCCGGCG AGGCCCATGC CCTGTCGAGG ATTGCATGCC
22801  ATGGCGCGCG ATTCGACGTT CGGCTTGGCG AGCCGGCCGT GCGCTGGCAC
```

Fig. 5 15/23

22851 TGCGCCCTGA CGCCTTGCGT GCACGGCGAC CTTGCCGATG GCGAGATGGA

22901 AAGCCTGCAA CTGCAATGGG CCGGGACGTA CATCGGCCTG ACGGTTCCGC

22951 GCGCGGCCGC GGCGGGATGG CTGGCGGCGC GCCTGCCCCG GTTTTCCGGC

23001 GTGGAGTTGC CGGAACCCAT TGCGGCGGCG GCCCTGGAGG CAATGCTGGA

23051 GGAGGTCTGT CGAGGCGTGG CCGGACTCGA CCAGCAAGGC CCGGTCCGCG

23101 TGGCGCGGCA AGGCGGGACG CCACCGGTCC AGCCGCATCG CTGGACCCTG

23151 ACGGTACGGG CGCCTGACGG TGGCGTCTGG CGCGCGGTAC TGGCGTGCGA

23201 CGCATGGGCC TTGCAAGCGG TCGCGGCGGC GCTGGATTCC GTTGCGCCTG

23251 CCGATGGTCG GGTCAATCCG GAGCGCGTGC CGGTCAGGTT GCGTGCCGAT

23301 GTCGGCGCGG CGTCCGTGAC CGCAGGCCAG CTGCGGACGC TGCGAGCGGG

23351 CGACGTCGTG TTGCTCGCGC AGTACCGGGT GAGCGATGCC GCAGAACTAT

23401 GGTTGTCGGC CGGACCCAGC GCGATCCGGG TACGGGCCGA GCATGCGTCT

23451 TTTCGTGTAA CTCAAGGTTG GACTCCCATC ATGACGGAAC CCGCGACACC

23501 TGACCCTGGC GAAACCCCGG CACAGGCCGA CGCGACGCTC GATACCGATC

23551 AGATACCCGT GCGCCTGACG TTCGACCTGG GCGAGCGCGA GTTCACGCTT

23601 GCGCAGCTGC GCAGCCTGCA TCCGGGCTGC ACGTTCGACC TCGAGCGGCC

23651 CATCGCCGAC GGGCCGGTCA TGGTGCGGGC CAATGGCCTG TTGCTGGGCA

23701 GCGGCCGGCT GGTCGACATC GACGGCCGCA TCGGCGTGGT ATTGCAGTCG

23751 GTCAGGCCTG GACTCGCATG AGCGATACCG ACCCCTTCAG CCTGGCCCTG

23801 TTTCTGGCGC TGCTGGCGCT GGTACCGCTC ATCGTCGTCA TGACCACGTC

23851 GTTCCTGAAG ATCGCCGTCG TGCTTGCCTT GGTGCGCAAC GCCCTGGGAG

23901 TGCAACAGGT ACCGCCCAAC ATGGCCCTGT ACGGGCTGGC GCTTATTCTT

23951 TCCGCGTATG TGATGGCGCC GGTCGTTCAC AGGATAGGCA CCGAGGTCCA

24001 GGCCTTGACC GCGCAAGCCG GGGAGTCCGG CACCGCCGCG CCGATGGCGC

24051 TGGACGCCGT GCTTGGCGTG GCCGAGCGAG GCGTGGGGCC GCTGCGGGCC

24101 TTCATGTTGC GCAACAGCCA GCCGGCCCAG CGTGATTTCT TCCTGCGCAC

24151 AGCGCGTCAT CTCTGGGGCG AGGAGGCATC GCGGGACCTG TCGGAAGACA

24201 ACCTGCTGGT ATTGACGCCC GCATTTCTGG TTTCGGAGCT GACCGCCGCA

24251 TTCCAGCTTG GCTTTCTGCT GTACCTGCCG TTCATCATCA TCGACCTCAT

24301 CGTATCGAAC ATTCTTCTTG CCATGGGAAT GATGATGGTT TCTCCCGTGA

24351 CGATCTCCAT GCCGTTGAAG CTGTTCCTGT TCGTCATGGT GGACGGCTGG

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24401  ACGCGCCTGA TCCAGGGCCT GGTGCTTTCC TATCGGTGAC CAGCATGCAA
24451  ACCCAAGACC TGGTTTCGTT CATGACACAG GCGTTGTACC TGGTGCTCTG
24501  GCTGTCGCTG CCGCCCATCG CCGTGGTGGC GATCGTGGGA ACGCTGTTTT
24551  CCCTGTTGCA GGCCTTGACG CAGGTGCAGG AGCAGACCCT GTCCTTCGCC
24601  GTGAAGCTGA TAGCCGTGTT CGCCACGCTG ATGCTGGCGG CCCGGTGGAT
24651  AAGCGCGGAA ATCTATAACT TCACGATTGC GGTGTTCGAT GCCTTTCATC
24701  GGATCCACTG AGCGGCCAAT CGATGCACAC GGAGTTCAAT TTCGTCGAGG
24751  CGAAGGTTTT CCTGGGAACG CTGGCCATGA CGCAACCGCG GATACTCACG
24801  GCCATGCTCT TTCTGCCGAT GTTCAACCGT CAGTTTCTGC CTGGTCCGCT
24851  GCGTTACGCC GTCGGCGCCT GTCTCGGGCT GATCGTGGTT CCCCAGCTGG
24901  CGCCGCAGTA TGCCGCGCTG GATATCGACT GGCCCCGGCT GCTGGCGCTG
24951  CTGGCCAAGG AGGCGATGGT GGGCATGTTC CTGGGTTGGC TGGCTGCCTT
25001  GCCATTCTGG ATCTTCGAGG CCATCGGCTT CGTCATAGAC AACCAACGGG
25051  GCGCCAGCCT GGGCGCTATC CTCAACCCCG CCACGGGCAA CGATTCGTCG
25101  CCCATGGGCA TTCTCTTCAA TCTGGGATTC ATGGTGTTCT TCCTGACGGC
25151  GGGCGGATTC GGGTTGTTCG CCACGATGCT GTATGACAGC TTCGGGTTGT
25201  GGAACATCTG GGCGTGGTGG CCGTCCATGC CCGCACAGGG CGCCGTGCGG
25251  ATGCTGGACC AGTTCAGTGG CTTTGCCGCG CGTGTCCTGC TGCTGGCCTC
25301  GCCGGCCATC GTGGCCATGT TCCTGGCCGA GCTGGGCCTG GCCCTGATCA
25351  GCCGCTTCGC GCCTCAACTG CAGGTGTTCT TCCTGGCTCT GCCGGTAAAG
25401  AGCGCGCTGG TGCTGTTCGT GCTGGTGCTG TACATGGCAA CGTTGTTCCA
25451  GTATGCAGGC GAAATCCTGG GTTCTGTGGG CCGGATCGTG CCGTTCCTGC
25501  ATTCAGCGTG GCCCGGCCCA TGAGCGGCGA GAAAACCGAG CGGCCCACCC
25551  CGAAGCGCCT GCGCGATTCC CGCGAGAAAG GCGAGGTCGC ACACAGCCGG
25601  GACTTTACCC AGACGGCGCT GATATGCGCC TTGTTCGGGC ACTTTCTGAT
25651  CAATGCCCCG TCCATTCTCG CGTCGCTGCG AGCGCTGATA CTGGCGCCGG
25701  CGGCCTTTGC CGACCAGGGG TTCGCCGTCG CATTGGGGCC CGTGCTGACG
25751  GAAATCCTCG ATCAGGCCGT CCGCGTGCTC GCTCCGCTGA TTCTCATCGT
25801  GCTTGGGGTG GGGATGTTCG CCGAATTCCT GCAGGTAGGC GTCGTGCTGG
25851  CGTTTCGAAA GCTCAAGCCT TCGGCGGAGA AACTGAATCC CGCCGGCAAT
```

Fig. 5 17/23

25901 TTGAAGAATA TCTTCTCGGC GCGCAACCTG ATGGAGTTCA TCAAGTCGGT

25951 ATGCAAGATC CTGTTTCTGG CGGTGTTGGT CACGTTGGTG ATACGGGATT

26001 CCTTGCAGCC GCTGATGGCC GTTCCCCATA GCGGGCTGGA CGGGTTGCGA

26051 ACGGGCGTAG GCCGCATTCT GCAGGTCATG GTCTGGAACA TCGGACTGGC

26101 GTACGGGGCG ATTTCGCTGG CGGACCTGGC CTGGCAGCGT TACCAGTATC

26151 GCAAAGGCTT GCGGATGAGC AAGGACGAAG TGAAGCAGGA GTACAAGGAG

26201 ATGGAAGGCG ATCCCCATAT CAAGCAGCAA CGCAAGCACC TGCACCAGGA

26251 GCTGATCATG CATGGCGCGG CGGCCCAGGT TCGCCGGGCG ACGGTGCTGG

26301 TGACCAATCC GACACACCTG GCCGTGGCCC TGTACTACGC GGCGGGCGAG

26351 ACGCCCTTGC CGCGCGTGCT GGCCATGGGG CAGGGAGCCG TGGCCGCTCT

26401 CATGGTCGAG GCCGCGCGCG ATGCCGGCGT GCCGGTCATG CAGAACGTCG

26451 CGCTGGCCCG CGCCTTGCAC GACCAGGCGG AGGTGGACCA ATACATTCCC

26501 GGCGAGTTGG TGGAGCCGGT GGCCGCGGTG TTGCGGGCGG TGCGCCAGGC

26551 ACTCAAGGAG CAGACATGAC AGCAACCATT CATCCCGATA TTGCCGATTA

26601 TGCGCGACGC CATGGCCTCG AACCCTCGGT CGACGCCGAT GGCGGGCTTG

26651 CCGTCCGGAT CGACGGACGG CATCGCGTCA GGTTGATCCC CGCCGAAGAC

26701 GGCATGCTGG TGTTGCGGGC GCGGCTGGCC GAGCTGCCCG ATGGGTGGCA

26751 GGCGCGCGCG GCGCAGTTGC GCCGGGCGGG CCTGCTGGCC AGCGCCATGG

26801 CCCCTGCGAC CGATGCGTAC TGCGGCATAG ACCAGGGCGA AACCGCGTTG

26851 TATCTGCACC AGCGCGTCGC ACCGGCCGGC AGTGCGCTGG CGGTGGACGA

26901 GGCGGTGGGC GAGTTCGTCA ATGCCTTGGC CACTTGGAAA AGGGCGATGG

26951 CGCAATGGCA ATAGGTCGGC TTGGGTATCT TGTCCGCGGC GCATGGGCCG

27001 GGGGTGTCAT GCTGTTGGCG GCCGGTAGCG CCTGGGCGGC GCCGAACTGG

27051 CCTTTGGCGC CGTATAGCTA CTACGCGCAG CAGCAGAGCC TGTCCGATGT

27101 GCTGCGCGAG TTCGCCGCAG GCTTCAGCCT GGCGTTGCAA CAGGGCAAAG

27151 GGGTGCAAGG CGTGGTCAAT GGGCGTTTCA ATGCGCGCAC ACCCACGGAG

27201 TTCATCGAGC GTCTCAGCGG CATCTATGGG TTCAACTGGT TCGTGCATGC

27251 CGGCACGCTG TATGTCAGCC GCACCAGCGA CGTGGTTACC CGCGCGGTGG

27301 ATGCAGCCGG CGCTTCGCCG TCGGCGTTGC GCCAGGCCTT GCTGCAACTG

27351 GGCATCCTGG ACGAACGCTT CGGATGGGGA GAGCTGCCGG CGCAAGGCGT

27401 GGCCATGGTG TCAGGGCCGC CGGCCTATGT CGCGCTGGTC GAGCAGGCGG

27451 TAGCGGCGTT GCCCAAGGGG GCCGGCAATC AGCAGGTGGC GGTGTTTCGC

27501 CTCAAGCATG CTTCCGTGAG CGACCGGGTG ATCCGTTATC GAGACCAGCA

27551 GGTAGTTACG CCGGGGATGG CCACCATGCT GCGCCAATTG ATCCTGGGGG

27601 CGGGGCCGGG CAACGACGCG GCGCTGGCCG CGGTGGCGGC GCCGCTGCGG

27651 GAAAATCCGC CGGTGTTCGG CGATGCGGCA GCTGACGGGA ACGCGCCGCT

27701 CGCTGGCGCA GCCCAGGCAG CCGGCCGGCG CCTGAGCGAG CCCAGCGTGC

27751 AGGCCGACAC GCGCCTCAAT GCCTTGATCG TGCAGGATAT TCCCGAACGG

27801 ATGCCAATCT ACCGTGCCCT GATCGAGCAG TTGGATGTGC CCAGCACCCT

27851 GATCGAAATA GAGGCCATGA TCGTGGACGT CAATACCGAT CTGGTCAACG

27901 AGCTGGGTGT CACCTGGGGG GCGCAGATCG GAACCACCAG CCTGGGCTAT

27951 GGCGATCTGG GGCTGCGTCC CGGCAACGGC CTGCCCGTGG ACGGCGCGGC

28001 GGCCGACCTG GCGCCCGGAA CCTTGGGGAT CAGTGTCAGT ACCCGGCTGG

28051 CGGCGCGCTT GCGTGCGTTG GAGTCGGACG GGCAGGCCAA TATCCTGTCT

28101 CAGCCGTCCA TCCTGACCGC CGACAACCTC GGCGCCATGA TAGACCTGTC

28151 GGATACCTTC TACATTCGCA CCCTGGGCGA GCGCGTAGCG ACAGTCACGC

28201 CTGTCACGGT GGGTACGTCG TTGCGTGTGA CGCCGCGCTA TATCGCCGCC

28251 AAGGGAGGAC GCCAGGTGGA ATTGGCGATC GATATCGAGG ACGGACGGGT

28301 CTTGCAGGAG TATCCCATCG ATGGTCTGCC CCGGGTTCGG AAAAGCAGCA

28351 TCAGCACGCT GGCGGTGGTG GGGGACGAGC AGACGCTGCT GATCGGCGGC

28401 TACAACAATC GCCGTGACGA AGAGCAGGTC GAGAAAGTGC CGCTGCTGGG

28451 AGATATCCCC GGCCTGGGGT TCTTGTTCTC GAGCAAGTCC CGGGCGGTAC

28501 AGCGCCGCGA GCGGCTGTTC CTGATCCGGC CGCGTGTCGT GGCTATCGAG

28551 GGCAAGCCGG TCTTCAGCCC CGTTGCGGGC ACGTCGCAGG TGTTCATGAG

28601 CACGGGTTGG GGCGGGCATG GCAGCAGCCT GAGCATTGCA CCCGGCGAGG

28651 GCGGGCATAC ACAAGTGCGT CATGATGCCC GGGCGGGCAG GCCGGTCCGG

28701 CTGGTGCCGG ATTCATTGCA TGTGGAGTAT GGCGAGGCGG GGGAGGCGTC

28751 GCCCTGAGCG TGGCCCGGGC AGGGGGGCTA CGGCACGCTG TCGTAGCCTC

28801 GTTCGCGCAG CGCTTCGCGC AAGGCACAGC GGGCGCGGGA AAGTCGGCTG

28851 CGAATGGTTC CTACAGGAAC CGAAAGCAGT GCGGCAGCCT CTTCATAGGA

28901 GAGTTCTTCC ACGCCGACCA TGAGGATCAC GTCGCGCATG CTTTCGGGGA

28951 GCTGCTCCAG CGCTTCGCGT AGCAAGCGCA TGCGTTGACG CTGCTCGGTC

29001 ACGGCTTCGG GGTTGGGCGC ACTGCATGGC ATGACACCCA GGCTGGCGTC

29051 GgAGGTGAAT TCATAACGGC GCTCTGGCGC ACGCGACAAG TGATTGCGGA

29101 CCAGATTGAG CGCGATGCCG TACAGCCAGG TGGAAAGCTG GGAGTCGCCA

29151 CGGAACGATT GATACGCGCG CGCCGCCTCG GCGAAAGCCT GCTGCGCAAG

29201 GTCCTCGACG TCGCTGCTGT GGCCGATGTG CTTGGCGATG AATCTCTGTA

29251 ATCGGCCGGC ATGTTCGGCT ACCAGGCTGC TCAGCAACTG CTGGTCCGAT

29301 CGCTCGGTGG CGATATCGGG ATATGCGTTG TCCGGTTTTT CGAGAGAAGC

29351 GGGAAGACCG GATGACGGGG GAGAAACCAT GCAAAGCGAT ACCAAGTGAA

29401 AGGGTGATAA TTCACGTCAC CAAGATACTG ACTGCCGGTT TTATCCGGCA

29451 GTTGTTAACT TCCGAAACTA ATGTCGGATC GCGGTCGCTA CCGGAGCATT

29501 CAGATACAAC GCGCTGAACG TCTTCCGTAA AACTTACGAC GCGACGTATG

29551 GGGACTACAC CAGGTGGTGC GCAAGGACCT CGCGCaaCCA TTCTTGCGCC

29601 GTATGGGCGG ATACCGGCGC CTTACCTGAT TGTGCGGCGG ATGCGAAGCG

29651 CTGCTGCATG CtTTCGTCGA TCGTGGCGCG CAACGTTCCG GATATCTGAT

29701 CGGgAGAGAC ACCGGTGTCG TGACAGACCT GTCGGAAGCC GaCGGCGTCG

29751 GCGCGGGCCG ACCAGGACAG GAAGGTAAGG AAATCCACGC CCTGCAGGGC

29801 GTGGCGTgAG GTTTCCGCCA TGTCGACCGC TCGCGTGACG ACGCGCGCCG

29851 ACAGCGATGT CGTCGGGACC GCGTGCAGAT CGAGCTCCTT GGCGACGGCG

29901 CGGGCGATTC CCGTGCCGTA GGCCAGCGCC AGCGCGCTGG AAAACATGAC

29951 AAGCGTGTCC TCGTCGGTGG CGACCCAGGA TACGTTGCGC CCTGACGGCG

30001 TCGTGCCGGA TGCGATGACC TGGAACTGCT CGCCTGCTTT GGTGACATAT

30051 AGGGTCTCTC CTTGGCTGGC GGCGCGCGCA AAGACATCAA GCTCCAAAGC

30101 AGGTAAAGCG GGTGGGATCT GGAAGTTCAT GCGGTGGGCC GTTGTCTCGA

30151 ATCCTTGGGT GTATCGCGTT CTACGGAGCG GGAACATGGA ATATGCACTG

30201 TGTGACGCTT TCGGCTCTTC GGTTCCATGC CGGCATGACA AACCCGACGG

30251 CATGCAGGCC TCGCTCCCGT CCGGCCACCG AGGCTCGCCT TGAACGCTGC

30301 GCAAAGTGTC GGCCATACGC TGGGCCTCGA GCTGGAAATG GTGGTGGCcT

30351 GTCGTGCGAC CGGGGCCAGC CATCCGGTGG CGCGCCATTT CGAAGCGCTG

30401 CGCAACTTGC GCCGTCAACG CGGAGAGTCC GTGCAGGAGT ACCGTCTGGA

30451 CGCGCGCCTG TGCGGGGGGT GGGCGGTCCC CATGGCCTGA GCGGGCTTGA

Fig. 5 20/23

30501 TAACGGCTAT AACCTGCTCG AAACCGCATT CGCGCCTGTG AACGGCGGCG

30551 CGGGAGGGCT GCGCCGACTG GCCGAAGCGG TGCGCCGCGA GCTGCGTGAC

30601 ACGCAGCTCG CCCTGGCCGC GGAGGGGGCG ATGCTGATCA ACGCGGCCGA

30651 GCACCCCGCC GCCAGCCTGG ATGCCGACTG GTACCGGCGA GTGCGGGTGC

30701 CGCGGCCCAT CTACGAGGAA CTCGTCGGCC AGCGAGGCTG GCTGCACCGG

30751 ATCGGGATAG ACGCCAAGGC ACAGAACAGC CCCTGCACGT CGGTTCCCGT

30801 GGCCATCGCC GCGCGCTGCC TGAACGTCGT GCTGGCGCTG GCTCCCGCGC

30851 AGATCGCCAT GTTCGCCAAC AGCCCGCTGG AGGCAGGGCG GGTGACCGGT

30901 CTCAAGGAAA ACCGCCTGAC CCTGTGGCCG CGCATGTTCC GAGGCGCGCG

30951 CTACCTGGGC GACGACCTGC TGCATCGCCT GCCTGCAAGG CCGTTTCGCG

31001 ATCTCGGCGA TTATTTCCGC TGGATGTTCG GCGGATTGAC CGCCAGCCGG

31051 GCGCTACCGC CGGGCGACGC TTGCGACTAC AAGAACGCCG ATGTGGCCTG

31101 CCTGGTGGGA GCCCCTTCGC TGGCAGAGTT CCTGTATGCG GGCGCGTGGT

31151 CCGCGCGAAA CCTGAATGAT GGCGGTTCCG TGCGTCTGGC CGCGCGCAGC

31201 GAACATTTCG TCTATTCGCA GTTCGCGCAG TTCCTGGACG CGCGTTGGCG

31251 CTACAGGATG CCGATTGTCC CCGCCTTGCC GGCGCTGTTG CGAGCCTGGG

31301 ACAGGCAGGG CGGCCTGGAA GCGCTGTTCG AGCAGGCCGG CGCGCAAGGC

31351 TACATCGAGG GGCGCGCGCC GGGCGCGGTA TTTGCCGATG CCGACTTGCT

31401 GAGCTCAGCC GGCGATGCAG TCGCGGCCAG TGCGCCGATG GCGGCGTCGG

31451 CGCTGCAATT GGGGCTGTTG CGCAATCTGC ACGACGCCGA GGCCCTGGTG

31501 AGGCGATGGG GCTGGCTGCG CTTGCGTGCG TTGCGCGATC GGGCCATCGC

31551 TTTGGCGTTG GACGATGCGC AGGTGCGCTG CCTTTGCCAA CAGGTCGTGG

31601 CGGTAGCCGA AGGCGGGCTG GCCGGCGACG AGCAGCAATG GCTCGATTAT

31651 GTGCGTTACG TGGTGGAAAC CGGCGAGACC GCCGCGGACC GCATGCTGCG

31701 CTTGTGGCGC CAGGCGCGCG GCACGCCTGA GATGCGCCGC GCACAGGCGT

31751 GCCGGCAGCG CGCGGTGCTG TCCTAGGATC CGGCACATTC CTTGCCAGGT

31801 CTCGTTGGCC AGGCCTGTCA GTGCTCCGCT TCGTACACCT CGTCGGCCGC

31851 CAGCAGAATA TCCACCGGTG GGTCGAAGCC GATGCGCCGC GCCGTTTCCA

31901 GATTGATGGC TATCTTGGCG GGGGCATTCC AGACCTGGCT GATGCTGCGC

31951 GGCTTTTCCC CATTGAAAAT GCGGGCAATG GTCTGGGCGT GGAACATGCC

Fig. 5 21/23

32001 TACGCTGGAG TAGTCCGCCT TGGCCAGGCT CATCAACAGG CCGGCCTTGA

32051 CCTCGTCGGA GCCTTGCATC GAGAAACTCG GCACGCGGGC GGCGCGCAGC

32101 AGCGCGGCGA GCTGCTTGAC GGACGTCGAG GTGATGCCCC GGTGCTCGGT

32151 GACGTaAAAG GCGTCGACTT CGcTCGACAG CTTCTGGTAG CAAGCCAGAA

32201 CGTTCTGGGT TGCCGTGGCG ATGGGGATGC CGGTCGCGCG TGCGTCGcAA

32251 CGCTTGACGG AGAAATCCAA TGCCGGCATT AGTGCGGCGA CCTTATCGAT

32301 GGCTGCGTAG GTGCGACCTG CTTCGGTGTC TTCGTAGACC AGTCCAAGCG

32351 TCTTGAaCGG CACGATGTCA TGGAGCAGCT GGATCTGCCG CTGGTAGTGG

32401 TCGGgCTGTA CCCGGGCATG CAGGTTGTCC TGGCCGCTGT CGGCCGCACT

32451 GGGTATGATC CGGGCGCTTA TcGGGTCGgT CGACGAGACG ACCACGGTGG

32501 GTACCGGCGT GCCCAGTTCG ACCATGTCCT GTCCAGCCCA GGTACCCATG

32551 GCGATGATCA GGTCGATGTC CTTGGCGCCA TGCAGGCGTg CCGCAACGGC

32601 TTCGCGCACG GCAGGCCGCA AGGcGGTGTC GAAGTTGCCG GGCTgCCACC

32651 ACGCATCGGG CaCGAACTCG ATGTAGTTGC TGCGGGCATG CGTGGCCAGG

32701 TAAAGCCAGG CCTTTCGCAT ATCGGTTATC TCGGGCATGT CGTCGATACG

32751 CAGCCATCCG AGTTGTTGCA ATGCGCGCGC GATCGCGTAG AGCGTGCGCG

32801 GATACTCCTC GTACTCGCCG CTACCCACAT AACCGATGCG CCATTTCCGG

32851 CCGGATGTAT GGGAGGGAGG CGGAAGGCGG GGCGAGGTAA GGGCGACGCC

32901 GGAGCTCAGG GCCGCGACAG GAGGAGGGCT GGATGCCGCC GCGATGGGCC

32951 ACGCGAGGCC AAGAAGCAGG GCGAGCGGGG CGAGTATTCC GGGGCGAAGG

33001 GTCATGGGCG ATGAATGGCG ATGATGGTGA GATCGTCGGA TTGTTCGGAA

33051 TCGGCGGCGA ATTCGCGTAG GTCGTGCAGA ATGTGCTCGA TGAGTTCGGC

33101 CGCTGCGTGC GGCGCGCCCT GCATCAGGGC GACCAGCCGC GGCAGACCAT

33151 ACTGGGCGCA GCCGCCGTGG ATGGCTTCGG TGACGCCGTC GGTAAACGCG

33201 ACCAGCGAGG TGCCGTTCGG CAAGGTGGTG CTCAGGGTGG AATACGCCTC

33251 GTTGTCCAGC ACGCCGCAGG CCGCGCCGCT GCTTCCTTGA AGCAGGCGGA

33301 CCTCGCCACG TTCGTCGATG AGCAGCGGCG GCGGGTGGCC GGCGTTGACC

33351 CAgGCCAGGG CGCCTGTTTC CGGGGTGAAG ACGCCTATCA GCAAGGTGAC

33401 AAACATCAGC TTGGGGTTGT TCTCGGCCAG ACGGTGGTTC ACCTTGGTGG

33451 CGATGGCGCC CGGGTCGTGC TCTTCTTCCG CCACGCTGCG TATCAAGGTC

33501 CTGACGATGG CCATGAACAG GGCCGCGGGC ACGCCTTTTC CGGATACGTC

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33551  GCCGATGGCA AAGCACAGAC GCCCGTCTGC CAGCACGAAG TAGTCGTAGA
33601  AATCCCCACC GACCTCCCGG GCCGGGTACA TGACGGCACG CAACTGGCTG
33651  CCGCGCGTGG CCGCATCGGG CAACGGCTGG GGAAGCAGGC CAAGTTGGAT
33701  GGAGCGGGCG ATGCTCAATT CGCTTTCGAG GCGTTCGCGG TTCGATATCT
33751  GCGCCATCAG GGCCCGCACA TTGTGGTGCA GCTGTTCGTT CATGAACAGG
33801  AACGATTCGG CGAGCTGTCC GACTTCGTCG CGCCGCCGGC GCGGCAGGCA
33851  TGCCACCGAC GGCGGAACCC GGATCGGCTC GGTGAGGTCC TGGGTGGGAA
33901  GCTGGCGAGC GTAGTTGCTC AGTTGCGCCA ACGGCCGGGC GATGCGCACC
33951  GCCACCACCC ATGCCAGCAT CAGCCCGGCC AGCAAGGTGG CGGCGAAGAT
34001  CAGTGCCTGC CGGCGCACCA GATTCTGTGC CGGGTCGGTC AGGTCCGGCT
34051  CGGGAACGAC ACCGATGATG GTCCAATGCA GCGGCTTGTA TCGCAGGGCG
34101  TCGATCTGCC AGGCGCTTTC GCCGTTGGTA AAGCGCAACG TCAGGCCGCG
34151  GGTAGACGAG ATTTCGGCAA GCATCGAATG CAATACCCGT CCCGATTCGA
34201  CGTCTGTCGA GTCCAGCAGC CGGGCGGCCG ATGGGGGTGG CGGCACGATC
34251  ACCGTGCCAT CGTCCGCAAC CACGAACACG AAACCATGGC GGCTGAGCCG
34301  CAGCTCCGAC AGGTTCCGGT CTATCGCGGC AATCATGTTG GCTTTCTGGG
34351  CGGCAACCTT GTCGATGATG GCTTGTGAGC TATCGGAGAT GGCGAGAACC
34401  CACTTCCACG CGGGGAAGTA CACGAAATAG GCGTGTCGCA TCTGGGCGGA
34451  CTCGTCCAGG GGAGACGGGT AGATGGCGAA GCCGCGACCG TCGTTGCGGC
34501  TTTCCTCGTA CATGGCGGCG GCGAGCGGCC GGCCCTTGAA GTCGCGGATC
34551  CCggagAGGt CCCGGTCGAn nnnnCGGGGG TTGGTGCTGG CCAGCACnnn
34601  nCCTTcCGCG TCATAGGCGA AGGCGACGCG GCGCGGTCCC AGGTCGAGAT
34651  GGTTCAGCCA GACACGCGCC ATGCCCTTGG CGGCGCCGGT AGTGACGTGT
34701  CCGCGCTCGG CCTGCGCGGC ATAGGCGTTC AGCACCGATG TCACGACCGC
34751  GCTGAGTTGG ATCAGTTGCC TGCGGCTTTC CCGGATGGTG CGGATCTTGT
34801  CGTCGAGCAG CGTGGACCAG CGCGTGTCGG TGTCACGAAC TACCAAGTCC
34851  ATGATGTTAC TGACGGCATG CAGTTCGTTC TTGATGATGT TGTTCGTGAC
34901  ATCGCGCTGG GTCACGAGCA TCACGACGAT GCCTACGAGT AGCAGCGTGG
34951  ATGCAATGAG CAGGAGGAAC TTCCCACGCA ATGAAAGCGG CAACTCTAGC
35001  CGGGGAGTAC GGCGCATGAA CATGAA
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Fig. 5 23/23